



FUNGAL DISEASES

*An Emerging Threat to Human,
Animal, and Plant Health*



Workshop Summary

**INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES**

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FUNGAL DISEASES

An Emerging Threat to Human, Animal, and Plant Health

Workshop Summary

LeighAnne Olsen, Eileen R. Choffnes, David A. Relman,
and Leslie Pray, *Rapporteurs*

Forum on Microbial Threats
Board on Global Health

INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The serpent adopted as a logotype by the Institute of Medicine is a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

Cover images: Front (upper): Little brown bats with white-nose syndrome, New York, photo courtesy of New York Department of Environmental Conservation; Front (lower): Yellow stripe rust on wheat, photo courtesy of Stephen A. Harrison, Louisiana State University Agricultural Center. Spine: The Panamanian golden frog (*Atelopus zeteki*), photo courtesy of Wikimedia Commons, photo by Brian Gratwicke, Smithsonian Conservation Biology Institute. Back: *Geomyces destructans*, shown in a false-color SEM image (fungus hyphae are yellow, green, and orange; spores are blue), image reprinted from Chaturvedi et al. (2010) Morphological and Molecular Characterizations of Psychrophilic Fungus *Geomyces destructans* from New York Bats with White Nose Syndrome (WNS). *PLoS ONE* 5(5): e10783. Doi: 10.1371/journal.pone.0010783.

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Willing is not enough; we must do.”*
—Goethe



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The Forum on Emerging Infections was created by the Institute of Medicine (IOM) in 1996 in response to a request from the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH). The purpose of the Forum is to provide structured opportunities for leaders from government, academia, and industry to regularly meet and examine issues of shared concern regarding research, prevention, detection, and management of emerging, reemerging, and novel infectious diseases in humans, plants, and animals. In pursuing this task, the Forum provides a venue to foster the exchange of information and ideas, identify areas in need of greater attention, clarify policy issues by enhancing knowledge and identifying points of agreement, and inform decision makers about science and policy issues. The Forum seeks to illuminate issues rather than resolve them. For this reason, it does not provide advice or recommendations on any specific policy initiative pending before any agency or organization. Its value derives instead from the diversity of its membership and from the contributions that individual members make throughout the activities of the Forum. In September 2003, the Forum changed its name to the Forum on Microbial Threats.

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Workshop Overview

FUNGAL DISEASES: AN EMERGING THREAT TO HUMAN, ANIMAL, AND PLANT HEALTH

*Will the blight end the chestnut?
The farmers rather guess not.
It keeps smouldering at the roots
And sending up new shoots
Till another parasite
Shall come and end the blight.*

—Robert Frost (1936)

*Fungi are the only group of organisms that have been
convincingly shown to cause extinction.*

—Arturo Casadevall (2010)

At the beginning of the 20th century, the American chestnut population counted nearly 4 billion trees. The American chestnut tree, once dominant in the forests of the Eastern United States, was decimated by an accidentally introduced and previously unknown fungal pathogen. Within a span of 40 years, this once abundant, iconic forest tree was all but annihilated by this microscopic fungus. In the middle of the 20th century, an epidemic of Dutch elm disease—a vector-borne fungal disease, also unknown to science at the time—ravaged the elm trees of North America, Europe, and England (Brasier and Buck, 2001). Together, these diseases rapidly and radically transformed the landscape of America's cities and forests (Money, 2007).

Fungal diseases of plants, animals, and humans have altered tree population diversity and forest ecosystem dynamics, devastated agricultural crops, triggered global population declines and extinctions in wildlife, and contributed to death and disability in humans. *Cryptococcus gattii* (*C. gattii*), a pathogenic fungus that emerged in 1999 on Vancouver Island, British Columbia, Canada, is causing a growing epidemic of human and animal infections and deaths (Galanis and MacDougall, 2010). Since its initial recognition, the pathogen has spread from Vancouver Island to mainland British Columbia and south into the Pacific Northwest of the United States. This fungal pathogen has been associated with 338 confirmed human infections and 40 deaths¹ in these regions, which represents the largest documented population of *C. gattii* infected people in the world (Datta et al., 2009a; Galanis and MacDougall, 2010). Bat white-nose syndrome (WNS) and amphibian chytridiomycosis² have caused massive population declines and threaten local extinctions of New World bat and amphibian species, respectively (Frick et al., 2010; Skerratt et al., 2007). By 2009, the geographic range of two virulent and highly aggressive strains³ of yellow “stripe” rust—first detected in North America in 2000—expanded to include major wheat-producing areas on five continents, threatening the global wheat supply (Hovmøller et al., 2010). The recent observation that a fungus (*Nosema* spp.), in combination with a DNA virus, might be associated with “colony collapse” disorder—a disease that has destroyed 20–40 percent of the honeybee colonies in the United States since 2006—underscores the direct and indirect impacts and ecosystem dynamics of fungal diseases in human, plant, and animal communities (Bromenshenk et al., 2010).

Fungal organisms interact with humans, animals, and plants in beneficial as well as pathogenic ways. A dozen fungal diseases are considered “life threatening” to humans. At the same time, human health has benefited immensely from fungal-derived antibiotics, such as penicillin (Blackwell et al., 2009; Buckley, 2008; Casadevall, 2007). Indeed, fungi are indispensable to life on this planet through their ability to break down complex organic matter and recycle essential nutrients back into the environment (Wainwright, 1992).

The fungal kingdom is among the most diverse kingdoms in the Tree of Life (Blackwell, 2011). Yet, fewer than 10 percent of fungal organisms have been formally described (Hawksworth, 1991, 2001). For the purposes of this chapter, the terms fungi, fungal, and fungus are used inclusively to describe all organisms traditionally studied by mycologists—including species that are now excluded from Kingdom Fungi (e.g., *Phytophthora* spp. which are members of Oomycota) or whose relationship to the fungal kingdom have yet to be determined (e.g., the

¹ As of December 2010.

² In this chapter, we will refer to this disease as amphibian chytridiomycosis and to the associated pathogen (*Batrachochytrium dendrobatidis*) as *Bd*.

³ *Puccinia striiformis* Westend. f.sp. *tritici* Eriksson.

microsporidia *Nosema* spp. and the newly discovered *cryptomycota*) (see Jones et al., 2011; Stajich et al., 2009).

Despite the extensive influence of fungi on economic well-being, as well as on human, animal, plant, and ecosystem health, the threats posed by emerging fungal pathogens are often unappreciated and poorly understood. On December 14 and 15, 2010, the Institute of Medicine's (IOM's) Forum on Microbial Threats hosted a public workshop on this topic in order to explore the scientific and policy dimensions associated with the causes and consequences of emerging fungal diseases. Through invited presentations and discussions, the workshop explored the environmental, host (plant, animal, and human), and pathogen-related factors influencing the emergence, establishment, and spread of fungal pathogens, as well as the impacts of these diseases on human and animal health, agriculture, and biodiversity. Workshop participants also considered and discussed opportunities to improve surveillance, detection, and response strategies for identifying and mitigating the impacts of these diseases in order to better prepare for future outbreaks. Convened in response to the perceived threat posed by emerging fungal diseases to human, animal, and plant health, this was the first workshop in the Forum's 15-year history that focused exclusively on fungal pathogens.

Organization of the Workshop Summary

This workshop summary was prepared by the rapporteurs for the Forum's members and includes a collection of individually authored papers and commentary. Sections of the workshop summary not specifically attributed to an individual reflect the views of the rapporteurs and not those of the Forum on Microbial Threats, its sponsors, or the IOM. The contents of the unattributed sections are based on presentations and discussions at the workshop.

The summary is organized into sections as a topic-by-topic description of the presentations and discussions that took place at the workshop. Its purpose is to present lessons from relevant experience, to delineate a range of pivotal issues and their respective challenges, and to offer potential responses as discussed and described by the workshop participants. Manuscripts and reprinted articles submitted by some, but not all, of the workshop's participants may be found, in alphabetical order, in Appendix A.

Although this workshop summary provides a description of the individual presentations, it also reflects an important aspect of the Forum's philosophy. The workshop functions as a dialogue among representatives from different sectors and allows them to present their beliefs about which areas merit further attention. This report only summarizes the statements of workshop participants. This workshop summary report is not intended to be an exhaustive exploration of the subject matter nor does it represent the findings, conclusions, or recommendations of a consensus committee process.

THE HIDDEN KINGDOM

Fungi are among the most evolutionarily and ecologically diverse organisms on the planet, comprising a kingdom of organisms that provide valuable ecosystem services through their decomposition of organic matter, symbiotic associations with numerous plant and animal species, and as food sources (Blackwell, 2011; Taylor et al., 2004). Initially thought by early taxonomists to be members of the plant kingdom, fungi are actually more closely related to animals than plants (Figure WO-1) (McLaughlin et al., 2009).

According to keynote speaker Arturo Casadevall, of the Albert Einstein College of Medicine, fungal organisms—in terms of sheer numbers of species—constitute the most successful kingdom in the tree of life. (Dr. Casadevall's contribution to the workshop summary report can be found in Appendix A, pages 177–188.) Yet fewer than 10 percent of the estimated 1.5 million species of fungi have been formally identified and described⁴ (Blackwell, 2011; Hawksworth, 2001). Forum Chair David Relman, of Stanford University, observed that, “We are blind to a lot of their biology and what it is that they spend most of their time doing and why and for whom. I think many in this room would agree that fungi are ignored and underappreciated.” This “blindspot,” he continued, “leaves us with fairly poor situational awareness: a relatively poor understanding of fungal biogeography—meaning their spatial distribution patterns—the factors that determine their distribution in space and time, and the factors that underlie their evolution, especially within short time-frames.”

Fungal Diversity

Existing as single-celled organisms, such as yeasts, or complex communities of filamentous mycelial networks covering hundreds of acres, fungi are ubiquitous in nature and display a dazzling array of sizes, shapes, and colors, including many that are bioluminescent (Figure WO-2) (Blackwell, 2011; Desjardin et al., 2010; Lutzoni et al., 2004).

The fungal life cycle is equally varied. Fungi can reproduce asexually or sexually through life cycles that range from simple to complex—including “dimorphic” switching between yeast and filamentous forms and the use of multiple host species (Blackwell et al., 2009). Spores⁵ are produced during the fungal life cycle and may be passively or actively dispersed through a variety of environmental media including air, water, wind, animals, and materials (Blackwell et al., 2009). Fungal growth, reproduction, spore production, and dispersal are also exquisitely sensitive to environmental conditions including temperature, humidity,

⁴ This number is considered by many to be an underestimate of the actual number of fungal species; see contributed manuscripts by Blackwell in Appendix A (pages 116–167).

⁵ Spores are well-protected structures that can survive in adverse environmental conditions, such as freezing or drying (better than mycelia and yeast cells), for months and even years.

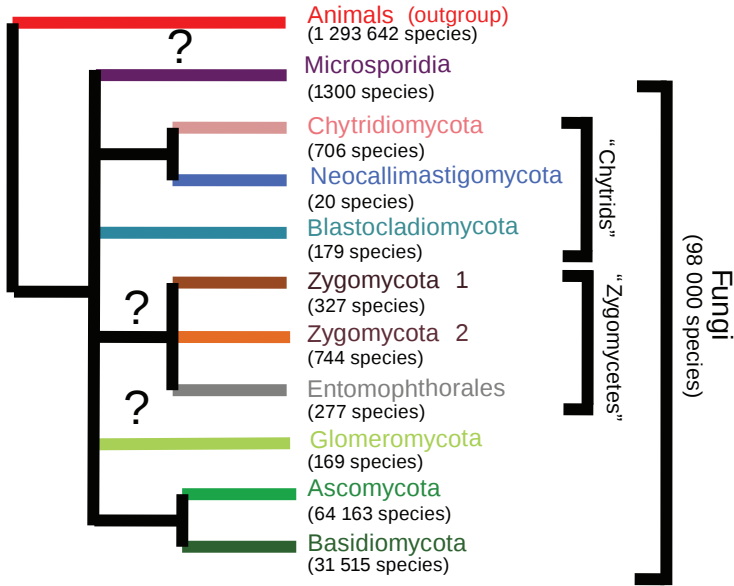


FIGURE WO-1 The fungal kingdom. The classification of species within kingdom Fungi continues to evolve. The diagram above provides an overview of some of the primary lineages of fungal organisms and the estimated number of species for each lineage. SOURCE: Blackwell (2010).

winds, and water (Bahn et al., 2007; Judelson and Blanco, 2005; Kauserud et al., 2008; Kumamoto, 2008).

Fungi are highly adaptable to new environmental niches including what might be considered “extreme” environments (Gostinčar et al., 2010; Le Calvez et al., 2009). Some have suggested the ability of fungi to access multiple strategies for reproduction contributes to why fungi are so “adept at adaptation.” Under different environmental conditions, fungal reproduction can maintain characteristics adapted to a particular environmental niche or generate genetically diverse offspring that can quickly respond to changing host or environmental factors (Heitman, 2006). (Dr. Blackwell’s contribution to the workshop summary report can be found in Appendix A, pages 116–167.) Keynote speaker Meredith Blackwell, of Louisiana State University, noted that scientists continue to find new species of fungi in a wide range of environments—from tropical and temperate forests to the guts of insects (e.g., Arnold et al., 2003; Gostinčar et al., 2010; Miller et al., 2001; Suh and Blackwell, 2006). These discoveries often reveal the unique capabilities of these microorganisms. As observed by Casadevall, some fungal species can survive and thrive in high radiation and other extreme environments. Zhdanova et al. (2000) reported extensive fungal growth on the walls



FIGURE WO-2 Diversity of fungal morphology. (A) Two flagellated fungal cells from the recently discovered group of fungi known as cryptomycota. This ancient group of organisms is thought to be distinct from other fungi because of the absence of a cell wall made of chitin; (B) asexual, spore-producing culture of *Cryphonectria parasitica* (chestnut blight fungus); (C–F) multicellular, spore-producing structures (fruiting bodies) are produced during the sexual phase of the fungal life cycle. Many fruiting bodies are familiar as mushrooms—including species that are consumed by humans as food: (C) *Morchella conica* (morel) and (D) *Crucibulum laeve* (bird’s nest fungus). Mushrooms of some species are known to be toxic or poisonous to humans: (E) *Amanita muscaria*. Fungal fruiting bodies can exhibit a wide range of shapes and sizes, including (F) the bioluminescent “shelf” fungus, *Panellus stipticus*; (G) Micrograph of *Phytophthora ramorum* chlamydospores; (H) SEM photomicrograph prepared from *G. destructans* culture isolated from bat tissue samples collected from Williams Hotel Mine; note curved conidia borne in whorls on septate hyphae; bar is 2 μ m. All images are pseudo-colored in Adobe Photoshop 9.0; (I) “fairy rings” in which mushrooms sprout along the outer edge of a sprawling, underground mycelial network. These networks (mycelia) have been known to cover several hundred acres. One of largest known mycelia has been estimated to encircle 900 hectares (3.4 square miles).

SOURCE: (A) Micrograph kindly provided by Meredith Jones, Exeter University; (B) photo by Kent Loeffler, provided by Alice C.L. Churchill, Cornell University; (C–F) Wikimedia Commons; (G) photo provided courtesy of Paul Reeser, Oregon State University; (H) Chaturvedi et al. (2010); (I) Wikimedia Commons.

and other areas of the shelter installed around the damaged unit of the Chernobyl nuclear power plant, including 37 species among 19 genera⁶; fungi are also known to inhabit high-radiation space environments and have even colonized the International Space Station (Dadachova and Casadevall, 2008).

The fungal pathogen responsible for sudden oak death and ramorum blight, *Phytophthora ramorum*, was only identified as a new species in 2000. Since then, according to speaker David Rizzo of the University of California at Davis, researchers have identified an additional 50 *Phytophthora*⁷ species. (Dr. Rizzo’s contribution to the workshop summary report can be found in Appendix A, pages 312–324.) As Rizzo observed, these new discoveries do not reflect recent fungal evolution, but are a reflection of the fact that “we just haven’t really been looking

⁶ Many of the species inhabiting the most heavily contaminated sites of the Chernobyl nuclear power plant were rich in melanin (a high molecular weight pigment). Dadachova et al. (2007) reported that radiation enhances the growth of melanized *Wangiella dermatitidis*, *Cryptococcus neoformans*, and *Cladosporium sphaerospermum* cells.

⁷ *Phytophthora* (“plant destroyer”) is a genus of approximately 100 species that includes several notorious plant pathogens, including *Phytophthora infestans*, which caused the Irish Potato Famine. *Phytophthora* species are oomycetes, which are fungus-like organisms in the kingdom Stramenopila.

for them.” Several other forest fungi that have caused major damage in the past, including the fungi responsible for chestnut blight and Dutch elm disease, were unknown to science until they started causing noticeable damage and die-off of forest and urban trees (Brasier and Webber, 2010).

Ecosystem Services⁸ and Interactions

The ability of fungi to process complex organic matter into essential nutrients (e.g., nitrogen, phosphorus) makes them indispensable members of virtually all ecosystems and “invisible” shapers of the world around us (Wainwright, 1992). The vast majority of described fungal species are saprophytic,⁹ surviving on dead plant matter and animal tissue (Blackwell et al., 2000). Fungi can be “free living”¹⁰ or form mutualistic, commensalistic, or parasitic relationships with plants, animals, and microbes—deriving benefits from and contributing to their living hosts (Blackwell et al., 2009).

Humans have used fungi as a direct source of food (e.g., truffles, mushrooms), as a leavening agent for bread, and in the fermentation of various food products, including, but not limited to, beer, wine, and soy products (Buckley, 2008). Some fungi contain psychotropic compounds that may be consumed recreationally or in traditional spiritual ceremonies, and they have been used for millennia for medicinal purposes (Capasso, 1998). The fruiting structures of a few species are highly valued in China for their purported medicinal benefits including as a “libido booster”¹¹ (Roach, 2011). Blackwell stated that since the early 1940s, fungi have been exploited for their life-saving antibiotics.¹² More recently, various enzymes and pigments produced by fungi have been used industrially and in the manufacture of a wide variety of products, including furniture, musical instruments, and clothing (Blanchette et al., 1992; Buckley, 2008; Keller et al., 2005). These organisms have been used extensively as biological pesticides to control weeds, plant diseases, and insect pests (Buckley, 2008). Blackwell observed that biomedical researchers have used certain species of fungi extensively as model organisms for genetic and other scientific research for decades.

Many fungi maintain close associations with their insect hosts. Blackwell discussed the symbiotic fungi that inhabit insect guts and are essential to the

⁸ Services provided by ecosystems that benefit humans and are necessary for a healthy planet like oxygen production, water purification, pollination, soil formation, and nutrient recycling. See www.conservation.org/resources/glossary/Pages/e.aspx (accessed on June 13, 2011).

⁹ Deriving nutrients from dead organic matter.

¹⁰ Not dependent on a host for survival.

¹¹ For example: A parasitic fungus, *Ophiocordyceps sinensis*, grows in the Tibetan Plateau in China and is highly valued for its “purported medicinal benefits,” including uses as “a treatment for cancer and aging and as a libido booster.” The nutty-tasting fungus is considered “fungal gold” because it can be sold for high prices in Chinese markets (see Roach, 2011).

¹² Other medicines such as the immunosuppressant cyclosporine A and statin drugs also are derived from fungi.

nutrition of many insects (e.g., Nardi et al., 2006; Suh et al., 2003, 2005). Fungi also are cultivated by fungus-farming termites and ants (Aanen et al., 2002; Currie et al., 2003; Dentinger et al., 2009; Munkacsy et al., 2004) (Box WO-1).

Not all fungal–insect associations are mutualistic. Blackwell described the parasitic but not usually pathogenic fungi in the order Laboulbeniales. She noted the reports of extreme host specificity exhibited by different species in this order—sometimes inhabiting only certain parts of the host insect (Weir and Beakes, 1995). Most laboulbenialean species are associated with beetles (Coleoptera), and flies (Diptera), but they are also associated with a diverse array of host species in other insect orders, mites and millipedes (Weir and Beakes, 1995).

Blackwell discussed a number of fungal–plant symbioses. She estimated that:

- Half of all ascomycetes (Phylum Ascomycota) are lichens [symbiotic associations between fungi and photosynthetic partners (algae)] (Lutzoni et al., 2001; Schoch et al., 2009);
- 90 percent of all photosynthetic plants have mycorrhizal associates (Ruehle and Marx, 1979); and
- 95 percent of all plants have fungal endophytes (Arnold, 2007; Rodriguez et al., 2009).

Endophytes—fungi that live inside the plant tissue but without causing any obvious negative effects—are less well known than other plant–fungal associations, but mycologists find them wherever they look (Arnold et al., 2003; Rodriguez et al., 2009). Numerous endophytic fungal infections have been observed in cocoa trees (*Theobroma cacao*) and they may play an important role in host defense by decreasing the damage associated with *Phytophthora* spp. infections (Arnold et al., 2003). To illustrate the complexity of these relationships, Blackwell noted interactions among the fungus *Curvularia protuberata*, the grass *Dichanthelium lanuginosum*,¹³ and a fungal virus. The grass infected with the fungus infected with “*Curvularia* thermal tolerance virus” provides thermal resistance benefits for the host plant. This tripartite relationship allows the grass to grow in the high-temperature soils of Yellowstone National Park (Márquez et al., 2007). Blackwell pointed to the red-cockaded woodpecker (*Picoides borealis*) as just one example of the many ways that fungi confer benefits to the health of ecosystems. These woodpeckers usually nest in trees infected with red heart rot (*Phellinus pini*) (Hooper et al., 1991).

¹³ Commonly referred to as Panic Grass.

BOX WO-1 The Fungal Gardens of Leafcutter Ants

Over the past 50 million years, a unique symbiosis has developed between attine (fungal growing) “leafcutter” ants and fungi in the Lepiotacea family. In what biologists consider the earliest form of agriculture, leafcutter ant colonies grow and meticulously maintain a specific fungal cultivar for food (Schultz and Brady, 2008; Wade, 1999).

Inhabiting forest ecosystems throughout Mexico and Central and South America, these ant colonies can number more than 8 million individuals. Foraging ants bring cut pieces of leaves back to the colony where they are broken down and fed to the fungus by worker ants (see Figure WO-1-1).

A second symbiotic relationship protects these fungal gardens. *Pseudonocardia* bacteria, which grow on the bodies of the worker ants, produce antibiotic compounds that prevent the growth of parasitic molds (Currie et al., 1999).



FIGURE WO-1-1 Leafcutter ants tending their fungal garden.

SOURCE: © Alex Wild.

For more information on leafcutter ants, visit the PBS video segment: “Ancient Farmers of the Amazon,” © WGBH Educational Foundation and Clear Blue Sky Productions, Inc., 2001, available at: http://www.youtube.com/watch_popup?v=RH3KYBMpxOU&vq=medium#t=11.

Or, use your smart phone to link directly to the video using the QR code at right:



Fungi as Pathogens

The longstanding utility of fungi to all life on earth has often been matched by their ability to directly or indirectly cause devastating disease in human, animal, and plant hosts. Fungi are the predominant pathogen species in plants, remarked Casadevall, and fungi can also cause disease in healthy humans and animals. Described by several workshop participants as “formidable pathogens,” many fungi can also endure adverse environmental conditions and thrive outside of their host (Casadevall, 2007).

Fungal pathogens in general execute a series of sequential steps in order to cause disease, remarked speaker Barbara Howlett of the University of Melbourne. (Dr. Howlett’s contribution to the workshop summary report can be found in Appendix A, pages 264–273.) These pathogens must:

- Recognize and attach to the host;
- Germinate, colonize, and derive nutrition from the host;
- Subvert host defense responses;
- Reproduce, exit, and disperse; and
- Find another host¹⁴ (Sexton and Howlett, 2006).

Very few fungal pathogens are able to cause disease in hosts from the plant and animal kingdoms; those that do are referred to as trans-kingdom pathogens (De Lucca, 2007).¹⁵ Fungi can also form different associations with different host types. For example, the fungus *Cryptococcus gattii* is pathogenic in animals including humans, but forms non-pathogenic associations with plants—which play an essential role in the maintenance of *C. gattii* spores in certain environmental niches (Bartlett et al., 2007; Xue et al., 2007). Once outside of a host, fungal pathogens of animals and plants often have different requirements for survival. Animal pathogens, noted Howlett, are often soil saprophytes that are free-living rather than obligate.¹⁶ In contrast, some plant pathogens can only survive on the tissue of a specific plant host(s).

¹⁴ For more information, see contributed manuscript by Barbara Howlett in Appendix A (pages 264–273).

¹⁵ Howlett noted two trans-kingdom pathogens during her remarks: *Fusarium oxysporum* f. sp. *lycopersici*, which causes vascular wilt in plants and is an emerging human pathogen (Ortoneda et al., 2004); and *Aspergillus flavus*, which infects corn and is an emerging pathogen in immunocompromised humans (Krishnan et al., 2009).

¹⁶ Capable of existing only in a particular environment; an obligate parasite cannot survive independently of its host (Science dictionary).

Fungal Pathogens of Plants

In addition to contributing heavily to annual losses in global crop production,¹⁷ fungal plant pathogens are associated with many notable episodes of human suffering and economic and ecological loss, including:

- **Irish Potato Famine:** The mid-19th-century epidemic of potato late blight in Ireland led to the Irish Potato Famine, which caused or contributed to the starvation and death of well over 1 million people and the emigration of another 1 million (Money, 2007; Vurro et al., 2010). At the time of the Potato Famine, one-third of Ireland's population of eight million was dependent upon the potato as a primary food source (Money, 2007) (Figure WO-3). See also Large (1965) and Woodham-Smith (1962).
- **Southern corn leaf blight:** The 1970 southern corn leaf blight epidemic led to the loss of 710 million bushels of corn—valued at more than \$1 billion at the time, or about \$5.6 billion in 2009 dollars (Tatum, 1971).
- **Dutch elm disease:** The impact of Dutch elm disease extends well beyond the death of 100 million mature elm trees in the middle of the 20th century. It not only transformed the landscape of cities and forests, but it has continued to alter associated ecosystem dynamics to this day through reduced food sources and nesting sites for wildlife, altered tree composition, and density (Loo, 2009; Money, 2007).

Fungal plant diseases have far-reaching health impacts that extend beyond the infected plant species—including, but not limited to, negative impacts on associated flora and fauna (Giraud et al., 2010; Loo, 2009). As the Irish Potato Famine illustrated, crop losses can have devastating impacts on populations that are heavily, or solely, dependent on a single food source for their caloric needs. Speaker Jim Stack of Kansas State University observed that with 59 percent of calories consumed by humans being derived from just four plant species (rice, wheat, maize, and potatoes), fungal diseases in these staple crops may catastrophically threaten local and global food security (Strange and Scott, 2005; Vurro et al., 2010). (Dr. Stack's contribution to the workshop summary report can be found in Appendix A, pages 273–296.)

Fungal Pathogens of Humans and Animals

Given the ubiquity and diversity of fungi, it is perhaps surprising that, of the nearly 1,400 recognized human pathogens, a little more than 20 percent (~ 325) are fungal, and fewer than a dozen are associated with “life-threatening” disease (Casadevall, 2007; Woolhouse and Gaunt, 2007). Historically, fungal diseases

¹⁷ Crop losses due to all pathogens (1988–1990) totaled \$33 billion for rice, \$14 billion for wheat, \$7.8 billion for maize, and \$9.8 billion for potatoes (Oerke et al., 1995; Rosenzweig et al., 2001).



FIGURE WO-3 Depiction of starving Irish children in 1847 potato famine; by *Cork* artist James Mahony (1810–1879).

SOURCE: Wikimedia Commons.

of humans have had a lower disease burden than bacterial, viral, or parasitic infections, although this disease burden may be changing. It has been noted that fungal diseases are increasing in incidence in the growing populations of immunocompromised human hosts (Romani, 2004). Once established, fungal diseases are often difficult to treat (Casadevall, 2007; Romani, 2004).

Disease in humans most often results from opportunistic¹⁸ infections (Shoham and Levitz, 2005). Only a few fungal diseases (e.g., coccidioidomycosis, histoplasmosis) are caused by “primary” fungal pathogens¹⁹ that induce symptomatic disease in otherwise healthy people (Casadevall, 2007; Cutler et al., 2007). The “apparent” resistance of humans to fungal disease may be a reflection of the host immune response, coupled with the high basal temperature of mammals, which often exceeds the thermotolerance²⁰ range for many fungi (Casadevall, 2005; Garcia-Solache and Casadevall, 2010; Robert and Casadevall, 2009).

Primary fungal pathogens of humans can also infect other mammals, such as domesticated livestock and companion animals. These diseases are generally not considered contagious and are acquired via inhalation of aerosolized infectious propagules²¹ from environmental reservoirs, such as soil or trees (Casadevall and Pirofski, 2007). According to speaker Luis Padilla of the Smithsonian Conservation Biology Institute, wildlife are also affected by opportunistic and primary fungal pathogens, but the epidemiology of these diseases in wildlife is not well understood. (Dr. Padilla’s contribution to the workshop summary report can be found in Appendix A, pages 296–312.) Two fungal diseases of wildlife, amphibian chytridiomycosis and bat white-nose syndrome, emerged rapidly and unexpectedly over the past several decades. These diseases are associated with unprecedented local and global population declines of amphibian and bat species, and pose serious threats to biodiversity and ecosystem stability (Frick et al., 2010; Wake and Vredenburg, 2008).

Fungal Pathogens as “Invasive Species”

“Fungi are the only group of organisms that have been convincingly shown to cause extinction,” Casadevall remarked, referring to the extinction of the land snail *Partula turgida* by a parasitic microsporidian fungus (Cunningham and Daszak, 1998). As Casadevall observed, this capacity for destruction may be

¹⁸ Resulting from pathogen entry via wounds or weakened state of the host, or as a disturbance of a normally benign host–fungus relationship.

¹⁹ Medically important fungi can be categorized as opportunists or primary pathogens. The opportunists rarely cause disease in an immunocompetent host whereas the primary pathogens do. For more information see: Cutler et al. (2007).

²⁰ Garcia-Solache and Casadevall (2010) define thermotolerance as the ability to grow at mammalian (37°C) and higher temperatures. Most fungi thrive in the range of 12°C to 30°C, but there are wide temperature tolerances among species, with some growing at temperatures as low as –10°C or as high as 65°C. See contributed manuscript by Casadevall in Appendix A (pages 181–188).

²¹ Spores or encapsulated yeast cells.

due, in part, to the fact that “when [fungal pathogens] get into an ecosystem—a vertebrate host, for example—they simply don’t care. They have no need for that host in order to go forward. They will take down every last member of the species.” In contrast, most newly introduced viral and bacterial pathogens in a naïve host eventually attenuate their virulence such that infection does not kill the host. Such adaptations are beneficial to both the host and pathogen in that the host survives and the pathogen avoids an “evolutionary dead end” (IOM, 2009). As noted by Casadevall and Pirofski (2007), the host independence of “environmental” microbes,²² including many fungi, may confer advantages that promote survival and virulence in other niches, including new ecosystems and novel host species.

The term “invasive species” is used to describe “non-native”²³ plants and animals that, when introduced to new environments, reproduce or spread so aggressively that they harm their adopted ecosystems (Carlton, 2004; Dybas, 2004). They compete with native organisms for food and habitat, act as predators or parasites of native species, and cause or carry diseases, often with devastating ecological and economic consequences (Pimentel et al., 2005). As observed by Morse (2004), infectious diseases represent another form of biological invasion—often arising “out of nowhere” with devastating effects.

Discussions during the workshop illuminated the capacity of many fungal pathogens to persist in environmental reservoirs and to readily adapt to new environmental niches and host species. Like invasive species, these fungal pathogens have been able to thrive in new environments and are changing the ecosystem in ways that are difficult to anticipate and even more daunting to prevent (Desprez-Loustau et al., 2007; Giraud et al., 2010; Rizzo, 2005). Given both the links and similarities between invasive species and many pathogenic fungi, it may be useful to view the origins of disease emergence, and the strategies deployed to prevent or mitigate the threats associated with fungal pathogens, through the larger lens of biological invasiveness.

FACTORS OF EMERGENCE

Diseases are categorized as “emerging” if their incidence²⁴ or virulence²⁵ has recently increased or if they begin to infect a novel host or population (WHO, 2010). As illustrated in Figure WO-4, disease²⁶ results from a complex interplay of interactions among the pathogen, host, and environment.

²² Microbes acquired from the environment (in contrast to acquisition from other living hosts) (Casadevall and Pirofski, 2007).

²³ Also called “exotic,” “alien,” and “nonindigenous” species.

²⁴ As used in epidemiology, the number of new cases of a disease that occur in a defined population within a specified time period; the rate of occurrence (IOM, 1992).

²⁵ The degree of pathogenicity of an organism as evidenced by the severity of resulting disease and the organism’s ability to invade the host tissues (IOM, 1992).

²⁶ A situation in which infection has elicited signs and symptoms in the infected individual; the infection has become clinically apparent (IOM, 1992). Some exposures to infectious disease-causing agents can also produce asymptomatic illnesses that can be spread to others.

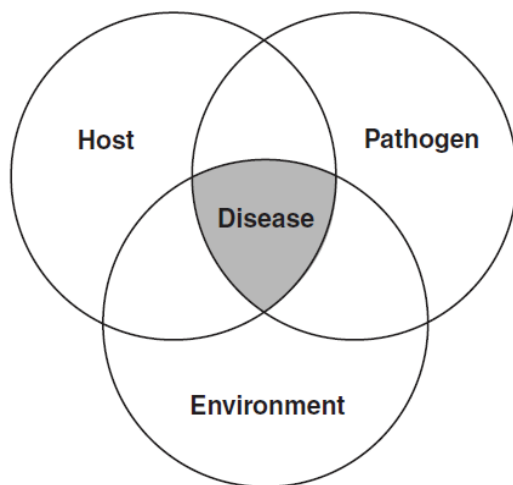


FIGURE WO-4 The epidemiological triad. The familiar “epidemiological triad” concept (host–pathogen–environment), as illustrated in the famous diagram of Snieszko (1974), neatly illustrates the complex interplay of factors that result in disease at the individual and population levels. The presence of a pathogen is a necessary, but not sufficient, cause of a particular disease (IOM, 2008b).
SOURCE: Snieszko (1974, Figure 1).

The range of factors identified as influencing the interactions between these elements (see Box WO-2) underscores the fact that exposure to a potential pathogenic agent is a necessary but insufficient condition for infectious disease emergence (IOM, 2003).

Significant factors for infectious disease emergence include the introduction of a pathogen into a new ecosystem or the disruption of an established ecosystem (IOM, 1992, 2003, 2010; Woolhouse and Gaunt, 2007). Such changes often expose immunologically naïve hosts to potential disease-causing organisms that have been released from the constraints imposed on them in their native environments (Woolhouse and Gaunt, 2007). Important catalysts for such disruptions and subsequent disease emergence include human activity, weather, and climate (Anderson et al., 2004; Daszak et al., 2000; Harvell et al., 2002; IOM, 2008a, 2010).

Anthropogenic and environmental factors play integral roles in the introduction and spread of many emerging fungal diseases. In recent years, the emergence of new plant diseases has been attributed to the evolution of hybrid pathogen species (Brasier, 2000). These hybrid species are thought to result from trade-mediated geographic redistribution of plants infected with the parental

BOX WO-2 **Factors in the Emergence of Infectious Diseases**

Thirteen factors of emergence of infectious diseases were elucidated in a 2003 Institute of Medicine report, *Microbial Threats to Health: Emergence, Detection, and Response*:

- Microbial adaptation and change
- Human susceptibility to infection
- Climate and weather
- Changing ecosystems
- Human demographics and behavior
- Economic development and land use
- International travel and commerce
- Technology and industry
- Breakdown of public health measures
- Poverty and social inequality
- War and famine
- Lack of political will
- Intent to harm

SOURCE: IOM (2003).

pathogens²⁷ (Brasier, 2000). As Rizzo noted, “for some of these tree pathogens, I don’t think there is anything extraordinary about the pathogens themselves.” Rather, “it is the movement of pathogens from one environment to another that seems to be driving much of the destruction.”

As described below, discussion at the workshop considered the influence of human activity and behavior, winds and weather, host susceptibility, and pathogen adaptation and change on fungal disease emergence.

Human Activity and Behavior

During the past century, human activities have dramatically influenced local environments and ecosystems, breaking down natural habitats and exposing new

²⁷ Stack explained that interspecific hybridization is another unexpected outcome of pathogen globalization. Two different (and previously isolated) fungal species mate and produce novel “hybrid” offspring. Fungal pathogens of plants produced by interspecific hybridization are more aggressive than either parental phenotype and may occupy a new host range (see Brasier, 2000). Stack noted that even in a single nursery, the normal process of taking care of plants, which includes watering, can result in a water splash that brings two different fungal species together in a single pot, where interspecific hybridization can occur.

hosts to infectious disease agents (Anderson et al., 2004; Brasier, 2008; Daszak et al., 2000). Travel, trade, migration, agricultural practices, and land use patterns have all contributed to increased opportunities for contact between introduced pathogens and naïve and susceptible host populations (IOM, 2010, and references within).

Speaker Matthew Fisher of the Imperial College London, remarked that migrating humans have been globalizing pathogens for thousands of years. (Dr. Fisher's contribution to the workshop summary report can be found in Appendix A, pages 355–367.) He pointed to the spread of *Coccidioides posadasii* that accompanied human migration between 5,000 and 10,000 years ago through North, Central, and South America as an example (Fisher et al., 2001). Stack agreed, noting that “global trade is not new; we have had 3,000 years of global trade.” He went on to state that, “what *is* new is the magnitude of trade in plants and plant products and the speed at which they move around the world” [emphasis added].

Trade, Travel, and Tourism

The increase in international transportation, travel, and trade associated with globalization in the 20th century has amplified the frequency of interactions between people, plants, animals, and microbes—providing novel opportunities for the rapid introduction, emergence, and spread of infectious diseases (IOM, 2010). The explosive growth of globalization—with dramatic increases in both the quantity and diversity of goods—has been enabled by a simultaneous decrease in travel time (IOM, 2010). Goods can be transported between most places in the world in less time than the incubation period for most infectious diseases (Cliff and Haggett, 2004; IOM, 2010). A study of factors associated with the emergence of diseases in crop plants demonstrated that the majority were spread via trade and travel (Anderson et al., 2004). Stack said this should not be surprising: In 2007 alone, the United States imported more than 48 million tons of agricultural products, only 1–2 percent of which were inspected for possible pathogens and other pests (Becker, 2009; Stack, 2010).²⁸

Local and global transportation of ornamental plants, trees, and timber also contribute to the introduction and spread of fungal diseases. The pathogens responsible for Dutch elm disease and chestnut blight were transported to America in shipments of beetle-infested timber imported from Asia and live chestnut trees imported from Japan, respectively (Money, 2007). Molecular epidemiological analyses of many *P. ramorum* isolates support the hypothesis that nursery plants infected with *Phytophthora ramorum* were the initial “source” for the epidemic of sudden oak death that began in California in 1995 (Mascheretti et al., 2008). *P. ramorum* has since emerged in the United Kingdom and Europe and now infects

²⁸ According to Stack (2010), each year, 12,000–14,000 potential pathogen and pest problems are intercepted during these inspections.

more than 100 plant species (Grünwald et al., 2008). Stack noted that potential pathogens can be transported in plants, plant associated material (e.g., soil), seeds, and objects manufactured using plant products, such as wooden instruments and packing materials.

Human spatial mobility has increased at least 1,000-fold in the past 200 years, with more people traveling faster, farther, and less expensively than ever (Figure WO-5) (Cliff and Haggett, 2004; Hufnagel et al., 2004).

Travelers are now able to easily explore once-remote areas that serve as both sources and sinks for emerging infectious diseases (Choffnes, 2008; IOM, 2010). Adventure travelers intrude on once-remote environments and often make contact with exotic wildlife, encountering microbes that have never before been recognized as human pathogens in the “developed” world (IOM, 2010). These ecotourists become unwitting vectors of disease when they bring these exotic infectious diseases back with them—on their person/clothing/luggage, etc.—when they return to their home countries. If the conditions are favorable, an introduced pathogen may persist and spread (Wilson, 2003). White-nose syndrome, which is currently decimating New World bat populations in the United States, may have been accidentally introduced by recreational cavers from Europe²⁹ (Wibbelt et al., 2010).

Infectious disease pandemics have also been associated with the legal and illegal trade in and transportation of animals (IOM, 2010; Karesh et al., 2005; Smith et al., 2009). Between 2000 and 2006, the United States traded approximately 1.5 billion animals, according to speaker and Forum member Peter Daszak of EcoHealth Alliance. (Dr. Daszak’s contribution to the workshop summary report can be found in Appendix A, pages 188–196.) These animals come from a wide range of species, and most animal imports into the United States come from emerging infectious disease “hot spots” (see Table WO-1) (Jones et al., 2008; Smith et al., 2009).

The international amphibian trade is thought to have contributed to the emergence and global spread of amphibian chytridiomycosis (Catenazzi et al., 2010; Daszak et al., 2003; Fisher and Garner, 2007; Schloegel et al., 2010; Weldon et al., 2004). Since the 1990s, this fungal disease has been implicated in the widespread population declines—including some local extinction events—of more than 200 species of frogs, toads, and salamanders (Fisher et al., 2009; Kilpatrick et al., 2009; Lips et al., 2006; Schloegel et al., 2006; Skerratt et al., 2007).

Speaker Ché Weldon of North-West University of South Africa noted the many points in the global amphibian trade pathway where traded species from different origins come into contact including collector-supplier facilities, breeding facilities, end-user facilities, etc. (Dr. Weldon’s contribution to the workshop summary report can be found in Appendix A, pages 355–367.) Weldon discussed two widely traded amphibian species—the African clawed frog, *Xenopus laevis*,

²⁹ Other possible explanations include the importation of horticultural soils from Europe.

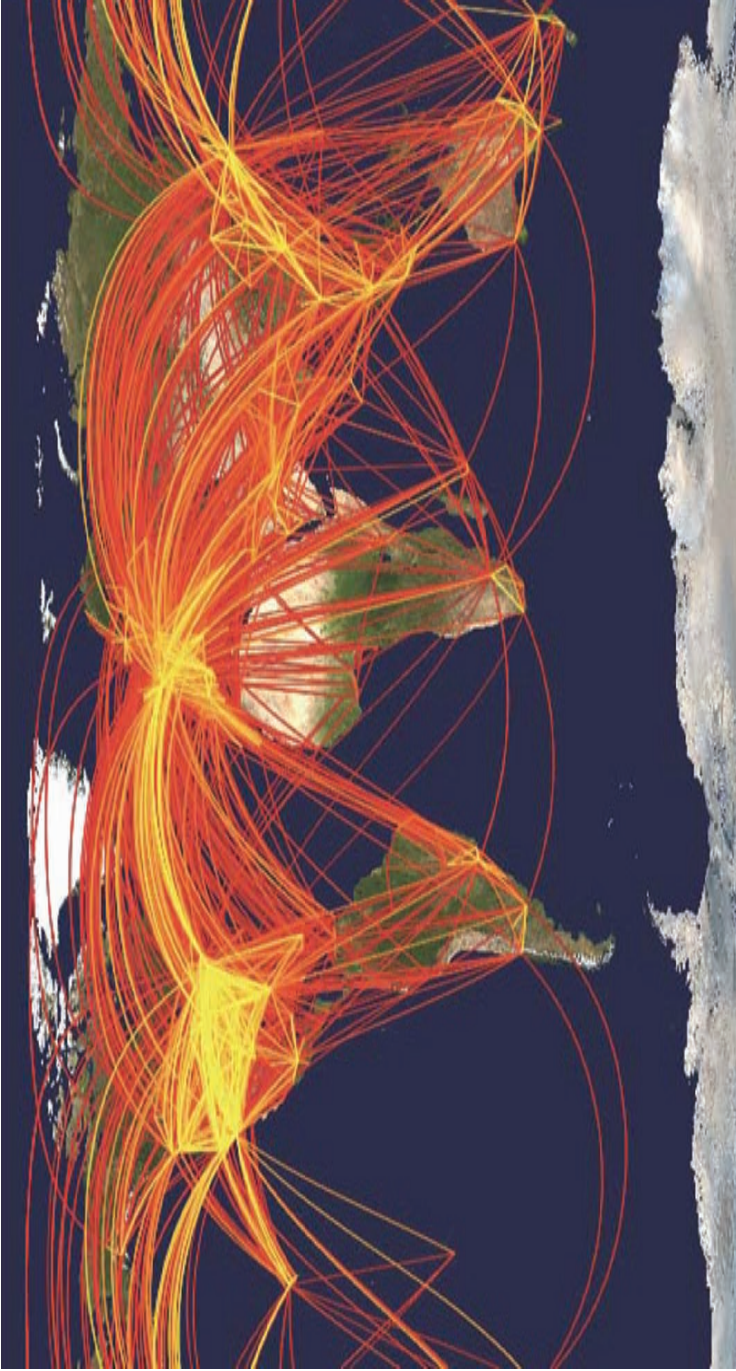


FIGURE WO-5 Global aviation network. A geographical representation of the civil aviation traffic among the 500 largest international airports in 100 countries is shown. Each line represents a direct connection between airports. The color reflects the number of passengers per day traveling between two airports, with the most intense traffic (25,000) noted in yellow.
SOURCE: Hufnagel et al. (2004).

TABLE WO-1 Number of Individual Animals Traded by the United States (2000–2006)

Class	Import	Export
Amphibia	27,631,172	1,594,961
Annelida	485,011	76,737
Arachnida	1,175,483	208,553
Aves	1,195,014	48,117
Chilopoda	4,358	274
Cnidaria	3,265,622	59,699
Crustacea	80,275,054	2,752,200
Diplopoda	13,926	1,218
Echinodermata	53,351	634
Insecta	469,606	88,686
Mammalia	184,682	32,879
Merostomata	60	0
Miscellaneous	5,430,083	154,195
Mollusca	3,187,671	555,829
Null	4,017,720	244,026
Pisces	1,316,977,591	138,404,653
Polychaeta	437	0
Reptilia	10,211,806	35984,895
TOTAL	1,458,805,947	180,207,556

SOURCE: Daszak (2010).

and the American bullfrog, *Rana catesbeiana*—that are “asymptomatic” carriers of the causative agent of amphibian chytridiomycosis *Bd. X. laevis* has been traded internationally since the 1930s (Weldon et al., 2007). Between 1998 and 2004 alone, more than 10,000 specimens of *X. laevis* were exported from South Africa to over 100 institutions in more than 30 countries worldwide (Weldon et al., 2007). *Rana catesbeiana* is one of more than 200 amphibian species in the international food trade, which altogether moves tens of millions of individual amphibians around the globe every year (Schloegel et al., 2010). He added that both of these species have now established feral populations in import countries, placing “native” species at risk of exposure to *Bd* (Weldon et al., 2007).

Winds and Weather

Along with anthropogenic introductions, wind and weather—including extreme weather events³⁰—are associated with the introduction, establishment, and spread of fungal diseases (Anderson et al., 2004). Because many fungal patho-

³⁰ Includes weather phenomena that are at the extremes of the historical distribution, especially severe or unseasonable weather (e.g., extreme heat or cold, tropical cyclones, tornadoes). http://en.wikipedia.org/wiki/Extreme_weather.

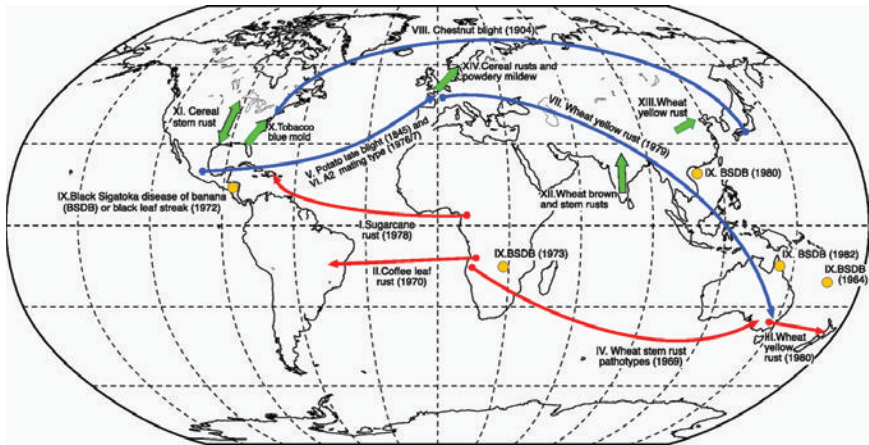


FIGURE WO-6 Selected dispersal events of fungal pathogens. Red and blue arrows indicate invasions of new territories (first year recorded in brackets). Red arrows indicate dispersal that probably occurred by direct movements of airborne spores (I, II, III, and IV). Blue arrows indicate pathogens that were probably transported to the new territory in infected plant material or by people and spread thereafter as airborne spores (V, VI, VII, and VIII). Orange circles indicate the worldwide spread of black Sigatoka disease of banana; the first outbreak on each continent is marked (IX). Green arrows indicate periodic migrations of airborne spores in extinction-recolonization cycles (X, XI, XII, XIII, XIV). SOURCE: From J. K. M. Brown and M. S. Hovmøller. 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* 29(5581):537–541, reprinted with permission from AAAS. Background map provided courtesy of Christopher Lukinbeal, University of Arizona.

gens are soil-associated, wind and other factors associated with soil disturbances can disperse spore-associated dusts into the air. Once airborne, spores may passively travel on the wind over great distances—often hundreds or thousands of miles—to new geographic areas and new host environments (Figure WO-6 [red]) (Brown and Hovmøller, 2002).

Aerial Dispersal—Winds and Extreme Weather Events

Limited dispersal of fungal spores carried by the wind is common and is considered a key factor in the local spread of some fungal diseases. Sporadic outbreaks of valley fever have occurred when spores of *Coccidioides* spp. are swept up from the soil and carried by winds to be inhaled by susceptible hosts. Long recognized as a threat to the health of military personnel stationed in arid regions of California, Valley Fever outbreaks have also been associated with land use changes and occupational or recreational exposures to dust (Chiller

et al., 2003; Crum-Canflone, 2007; Warnock, 2006). In 1994, the magnitude 6.7 Northridge earthquake led to an outbreak of valley fever in southern California (Figure WO-7A) (Schneider et al., 1997). More recently, the massive dust storm that swept through Arizona on July 5, 2011 is predicted to cause a similar increase in cases of valley fever (Chan, 2011) (Figure WO-7B). Speaker John Galgiani,³¹ of the University of Arizona, explained that even small winds or soil disturbances can easily loft spore-laden dusts into the air. (Dr. Galgiani's contribution to the workshop summary report can be found in Appendix A, pages 196–207.) Galgiani further observed that inhalation of a single spore “at the right time” can cause disease. Approximately 40 percent of infected persons develop symptoms, which initially manifest as pneumonia (i.e., cough, chest pain, fever, and weight loss); fatigue; bone and joint pains (“desert rheumatism”); or skin rashes (Hector and Laniado-Laborin, 2005; Tsang et al., 2010). While dust storms and environmental disturbances are clearly an important driver of the spread of *Coccidioides* spp., Galgiani said that simply living in an endemic region,³² without any direct contact with the soil, puts one at risk of exposure. Yet the fungus is only sparsely distributed. Galgiani noted that, “you can do a lot of desert digging and disrupting and not even be close to the fungus.”³³

Airborne spore dispersal may also synergize with intercontinental trade and travel to rapidly spread diseases between and within continents (Figure WO-6 [blue]). Yellow rust (*Puccinia striiformis* f. sp. *tritici*) is believed to have been introduced into Western Australia from southern Europe, in 1979, as an adherent spore on an air traveler's clothing (Wellings, 2007). Once introduced into Australia, the pathogen spread across Australia's wheat belt and into New Zealand via wind dispersal (Brown and Hovmøller, 2002). Indeed, winds allow many agriculturally important fungal plant diseases to gradually expand their geographic range (Brown and Hovmøller, 2002).

Pandemics caused by intercontinental aerial dispersal of spores can and do occur—often facilitated by hurricanes and other extreme weather events. Examples include:

- Sugarcane rust (*Puccinia melanocphala*) is believed to have been introduced from West Africa into America by cyclonic winds (Figure WO-6 [red]) (Brown and Hovmøller, 2002).

³¹ Dr. Galgiani is also Chief Medical Officer at Valley Fever Solutions, Inc. which has licensed the development of nikkomycin Z as a treatment for valley fever from the University of Arizona.

³² Although widely perceived as endemic to the southwestern United States, Galgiani observed that the endemicity of the disease extends through Mexico into Central and some parts of South America (Tsang et al., 2010).

³³ It has been suggested that this spotty distribution is a result of the abundant fungal sporulation that may accompany fungal decomposition of infected animal remains (whether or not fungal infection was responsible for an animal's death) (Sharpton et al., 2009).

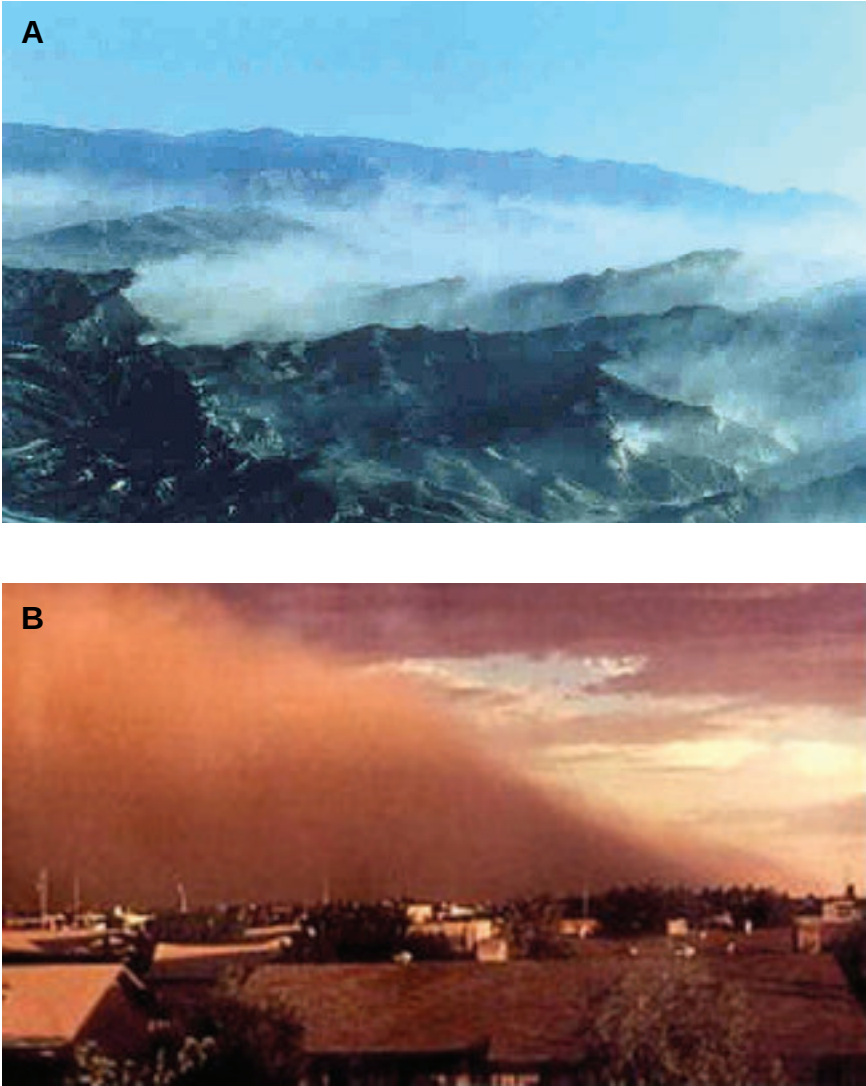


FIGURE WO-7 Environmental disturbances and dust storms contribute to the dispersal of fungal spores. (A) Dust from landslides caused by a 5.6 magnitude aftershock of the 1994 Northridge earthquake blows out of the Santa Susana Mountains into the Simi Valley. An outbreak of valley fever occurred in the Simi Valley following the January 17, 1994, 6.7 magnitude Northridge earthquake. (B) The leading edge of a violent dust and sand storm (known as a haboob). The wall of dust and sand that swept through Arizona on July 5, 2011, was estimated to be more than 50 miles wide. The storm travelled over 150 miles and reached peak heights of 8,000 to 10,000 feet.

SOURCE: Photos courtesy of Tom Freeman; National Oceanic and Atmospheric Administration.

- Coffee leaf rust (*Hemileia vastatrix*) may have been transported via transatlantic winds between Angola to Bahia in Brazil in 1970 (Figure WO-6 [red]) (Brown and Hovmøller, 2002).
- Asian soybean rust was brought into the United States from South America by Hurricane Ivan in 2004 (Schneider et al., 2005).

Some scientists are concerned that the frequency and duration of such extreme weather events could increase with global climate change, which in turn could influence the incidence and intensity of fungal disease outbreaks (Garrett et al., 2006; Greer et al., 2008).

Temperature, Humidity, and Climate Change

Like most microorganisms, fungi are highly sensitive to changes in weather and climate³⁴—particularly temperature, humidity, and wind—that can directly influence their growth, spread, and survival (Harvell et al., 2002). One of the most tragic outcomes of a weather-induced fungal disease outbreak was the Irish Potato Famine, in which a sustained pattern of cool, rainy weather enabled the emergence and spread of the “fungus-like” oomycete,³⁵ *Phytophthora infestans*, the causative agent of potato late blight (Fry and Goodwin, 1997; Large, 1965; Woodham-Smith, 1962). In 1845 and 1846, late blight led to yield reductions of 40 and 90 percent, respectively, in the potato—at that time Ireland’s staple food crop (Money, 2007). As previously noted, the resulting “Great Famine” led to the death of more than 1 million and the emigration of over 1 million more Irish people, primarily to the United States (Strange and Scott, 2005; Vurro et al., 2010).

When combined with reduced genetic diversity in the host plant, weather can contribute to a “perfect storm” for a devastating agricultural disease epidemic (Rosenzweig et al., 2001; Vurro et al., 2010). Unusually warm, moist weather, coupled with a wholly susceptible host, provided the ideal conditions for the emergence and spread of *Helminthosporium maydis* (also known as *Cochliobolus heterostrophu* and *Bipolaris maydis*), the causative agent of Southern corn leaf blight (SCLB) (Rosenzweig et al., 2001). Over the course of the 1970–1971 growing season, the SCLB epidemic spread from the tip of Florida up to Alberta, Canada, destroying a significant proportion of the corn crop in its path (Ullstrup, 1972). Yield reductions were most severe in the southern states, with many farms

³⁴ As explained on the National Aeronautics and Space Administration website (www.nasa.gov), the difference between weather and climate is a measure of time. Weather is the state of the atmosphere over a short period of time; climate is how the atmosphere “behaves” over relatively long periods of time.

³⁵ As noted by speaker David Rizzo, *Phytophthora* spp. is not a “true fungus”; it is an oomycete or “water mold” that belongs to the Kingdom Stramenopila (a major eukaryotic group that includes diatoms and brown algae, and is distinct from plants, fungi, and animals). Like fungi, oomycetes “exhibit filamentous growth, produce sexual and asexual spores, and can feed on decaying matter or be obligate parasites of plants” (Kliejunas, 2010).

experiencing total crop loss. Average yield loss in the Corn Belt states³⁶ was 20–30 percent, with some parts of Illinois and Indiana reporting yield losses of 50–100 percent (Ullstrup, 1972). In the 1970 season alone, the SCLB epidemic led to the loss of 710 million bushels of corn—valued at more than \$1 billion at the time (or about \$5.6 billion in 2009 dollars) (Tatum, 1971).

Compton Tucker, of the National Aeronautics and Space Administration (NASA) Goddard Space Flight Center, presented data from a variety of satellite and ground sources³⁷ documenting increases in global temperatures worldwide, as well as changes in the atmospheric concentration of carbon dioxide. (Dr. Tucker's contribution to the workshop summary report can be found in Appendix A, pages 324–342.) He also explained how general circulation models, which simulate the atmosphere, accounting for wind, humidity, clouds, temperature, composition of the atmosphere (e.g., presence of trace gases), and other weather-related variables, can be used to predict where on the surface of the earth (both land and water) temperature and precipitation levels are likely to change.³⁸ According to Tucker, these models predict that over the next century, average surface temperatures will increase by 2–5°C, and regions of the world will get wetter or drier (Figure WO-8).

Fungal diseases are influenced by weather fluctuations and display “seasonality”—suggesting the possible influence of long-term climate changes (IOM, 2003, 2008a; Rosenzweig et al., 2001). Stack noted that the onset of potato late blight has been occurring earlier and earlier over the past 20 years in some regions of the world and has resulted in more severe losses and greater mitigation challenges (Hannukkala et al., 2007). In part, this is due to changing temperatures and increased frequency of precipitation (Hannukkala et al., 2007).

Stack observed that modeling studies predict many negative impacts on plant health in response to climate change, including shifts in the range, timing, and severity of fungal diseases of plants³⁹ (Jeger and Pautasso, 2008; Pautasso et al., 2010). A 3°C increase in temperature, for example, is anticipated to alter the phenology⁴⁰ and conditions of the host species enough to result in expansion of the geographic range of *Phytophthora cinnamomi*, which has already decimated forests across southeastern Australia (Lonsdale and Gibbs, 1996). An enormous effect is predicted for the severity of phoma stem canker (*Leptosphaeria macu-*

³⁶ The area in the Midwestern United States, roughly covering western Indiana, Illinois, Iowa, Missouri, eastern Nebraska, and Eastern Kansas, in which corn (maize) and soybeans are the predominant field crops (Encyclopedia Britannica: eb.com).

³⁷ These data include NASA satellite data on solar irradiance (i.e., energy output of the sun); National Oceanic and Atmospheric Administration, NASA, and other surface data on land and ocean temperatures worldwide; U.S. military and other satellite and ground data on sea ice; sea-level data; NASA gravity data; and data on the atmospheric concentration of carbon dioxide and other components of the atmosphere.

³⁸ See also contributed manuscript by Tucker in Appendix A (pages 324–342).

³⁹ See contributed manuscript by Jeger in Appendix A (page 273–296).

⁴⁰ The scientific study of cyclical biological events, such as flowering, breeding, and migration.

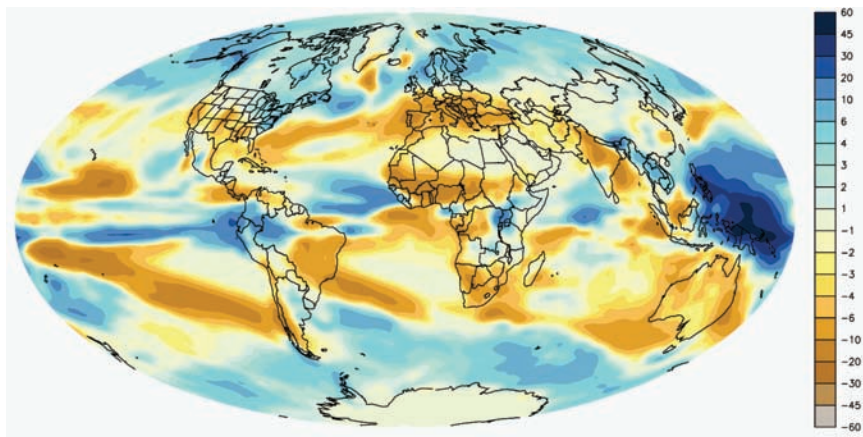


FIGURE WO-8 Change in precipitation between the 1971–2000 average and the 2091–2100 average in inches of liquid water/year.

SOURCE: Geophysical Fluid Dynamics Laboratory, National Oceanic and Atmospheric Administration.

lans) on oilseed rape, with many regions of the United Kingdom expected to experience a 40–50 percent yield loss by 2050 (Butterworth et al., 2010).

Modeling studies predict that it is not just the plant pathogens themselves that are likely to be impacted by continued climate change, Stack observed, but host species as well (Loustau, 2006; Pautasso et al., 2010). Stack remarked that while modeling studies forecast climate change effects on the distribution or severity of many fungal plant pathogens, for most crop plants the future is uncertain—both with regard to plant disease occurrence and the associated impacts on food security.

Host and Pathogen Characteristics

Whether caused by anthropogenic or natural forces, the mere introduction of a fungal organism is a necessary but insufficient condition for infectious disease emergence. Or, as viewed through the lens of biological invasion: Not all introduced species become “invasive.” Indeed, when introduced into new environments, invasive species become quickly established and spread in a new environment, while many other introduced organisms do not. As discussed at the workshop and summarized below, host and pathogen characteristics are important determinants for whether fungal pathogens will “thrive” in a new host or environment. For many emerging fungal pathogens, environmental factors have the greatest influence on the interactions between a naïve host and an introduced pathogen.

Host Defenses in Plants and Animals

As discussed previously, fungal diseases of plant or animal hosts involve several common steps (see “Fungi as Pathogens”). When it comes to host defenses, remarked Howlett, animals and plants have several important similarities and differences. Basal innate immunity⁴¹ is an important defense system shared by organisms that infect plants, animals, and insects, with immunity activated by recognition of pathogen-associated molecular patterns (PAMPs) (Nünberger et al., 2004). Other plant defense systems include a complex physical barrier (a thick and impervious cuticle and cell wall), a repertoire of pathogen-specific resistance genes, and systemic acquired resistance (i.e., if one leaf is infected and the plant does not die, the plant mounts a strong immune defense in the event that another leaf is infected later). By contrast, animals have a less complex physical barrier (i.e., the skin and respiratory surface) and distinct innate immune system components (with the complement system and phagocytes and other circulating cells) as well as a battery of adaptive,⁴² antibody-mediated, immune system defenses (i.e., T and B cells) (Sexton and Howlett, 2006). “Most serious human fungal diseases occur in immunocompromised hosts,” noted Howlett, suggesting that “the mammalian immune defense system is very effective.”

Fungal Disease and the Mammalian Immune System

Fungal disease in humans usually reflects some underlying immune dysfunction (Holland and Vinh, 2009). Speaker Steven Holland of the National Institute of Allergy and Infectious Diseases noted several examples of fungal diseases in otherwise healthy individuals that were ultimately associated with previously unknown primary immune disorders.⁴³ (Dr. Holland’s contribution to the workshop summary report can be found in Appendix A, pages 248–252.) Holland described a healthy and young individual with no previously recognized immunodeficiency who presented to an emergency department with acute shortness of breath that rapidly progressed to severe respiratory distress. The woman was eventually diagnosed with chronic granulomatous disease (CGD).⁴⁴ Infection by the ubiquitous fungus, *Aspergillus fumigatus*, probably occurred when she was handling soil and plant debris and led to the onset of symptoms (Siddiqui et al., 2007). Holland also reviewed the discovery of rare genetic immune deficiencies that

⁴¹ Immune response (of both vertebrates and invertebrates) to a pathogen that involves the preexisting defenses of the body (e.g., barriers formed by skin and mucosa, antimicrobial molecules and phagocytes). Such a response is not specific for the pathogen (Alberts et al., 2002).

⁴² Response of the vertebrate immune system to a specific antigen that typically generates immunological memory (Alberts et al., 2002).

⁴³ See contributed manuscript by Holland in Appendix A (pages 248–252).

⁴⁴ CGD is characterized by recurrent bacterial and fungal infections and inflammatory complications (Holland, 2010; Holland and Vinh, 2009).

underlie two serious diseases associated with fungal infection: Job's syndrome⁴⁵ and severe coccidioidomycosis (Buckley et al., 1972; Davis et al., 1996; Holland et al., 2007; Vinh et al., 2009).

The incidence of opportunistic fungal infections⁴⁶ has increased recently and is associated with the growing populations of vulnerable, immunocompromised individuals (e.g., people living with HIV/AIDS, recent organ transplant recipients) (Romani, 2004). In Casadevall's opinion, the period since the 1950s should be viewed as a transition decade in which "fungi become more important to human health" (Figure WO-9).

In the 1950s, only about 100 reported cases of disease were caused by *Cryptococcus neoformans*, Casadevall observed; today, there are about 1 million cases worldwide, mostly among persons with HIV/AIDS (Park et al., 2009). The yeast infection caused by *Candida* spp. was also uncommon until the 1950s. Many have associated the increase in *Candida* infections to the increased number of immunocompromised individuals (Dixon et al., 1996). Casadevall speculated that this may also be linked to the introduction of antibiotics, which altered the microbial flora in the human host.

Forum member Fred Sparling of the University of North Carolina, Chapel Hill, remarked "that there was significant cryptococcal disease⁴⁷ in the pre-HIV era," and that he continued to observe the disease in apparently healthy individuals. Holland agreed and noted that he expected that "we may find new mechanisms for susceptibility that might not be 'Mendelian,'⁴⁸ because it is not familial, but something that comes on, typically, in adulthood."

Casadevall considers host immune status in humans so important in the development of fungal disease that, in his opinion, fungal virulence can only be properly defined as a function of it. Casadevall went on to explain that pathogenicity is not an invariant, absolute quality in an infectious disease agent, but that the pathogenicity of a microorganism varies depending on the host and over time (Casadevall, 2007). He reviewed the "damage response framework"—illustrated in Figure WO-10—that was developed by Pirofski and Casadevall as a way to illustrate these concepts (Casadevall and Pirofski, 2003; Pirofski and Casadevall, 2008).

Host damage can derive from either the pathogen (e.g., among immuno-

⁴⁵ A rare, inherited disease associated with abnormalities of the skin, sinuses, lungs, bones, and teeth. People with this condition have chronic and severe skin infections (also known as hyper immunoglobulin E [IgE] syndrome). MedlinePlus: <http://www.nlm.nih.gov/medlineplus/ency/article/001311.htm>.

⁴⁶ These opportunistic fungal diseases include invasive aspergillosis and aspergilloma (*Aspergillus* spp.), invasive fusariosis (*Fusarium* spp.), *Pneumocystis* pneumonia (*Pneumocystis jirovecii*), and invasive candidiasis (*Candida* sp.) (Nucci and Marr, 2005; Pfaller and Diekema, 2010).

⁴⁷ Disease caused by *Cryptococcus neoformans* or *gattii* infection.

⁴⁸ A single gene disorder caused by a defect in one particular gene, and characterized by how they are passed down in families. MedlinePlus: <http://www.nlm.nih.gov/medlineplus/ency/article/002048.htm>.

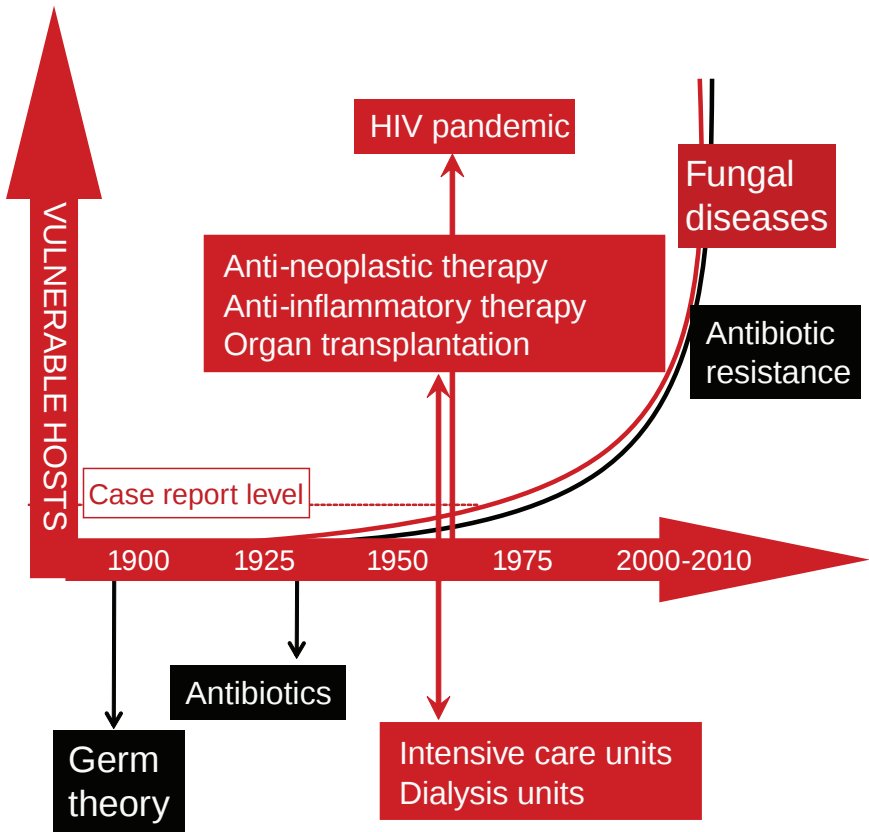


FIGURE WO-9 Incidence of systemic fungal disease has increased since the 1950s. Over the same period, the use of medical technology and the HIV/AIDS pandemic have led to an increased number of immunocompromised individuals. The emergence of systemic fungal disease in humans is considered by many to be a 20th-century phenomenon.
SOURCE: Adapted from Casadevall (2010).

compromised individuals with weak immune systems) or the host (e.g., among healthy host individuals whose microbial flora has been disturbed by antibiotic use, triggering a disproportionately strong immune response) (Casadevall and Pirofski, 2003). This model predicts that not just immunocompromised individuals are at risk of disease from fungal infection(s), but also healthy hosts who mount a disproportionately strong immune response (Casadevall and Pirofski, 2003).

Casadevall also suggested that there may be additional “subtle” effects of fungal infection that we are just beginning to observe on a population level.

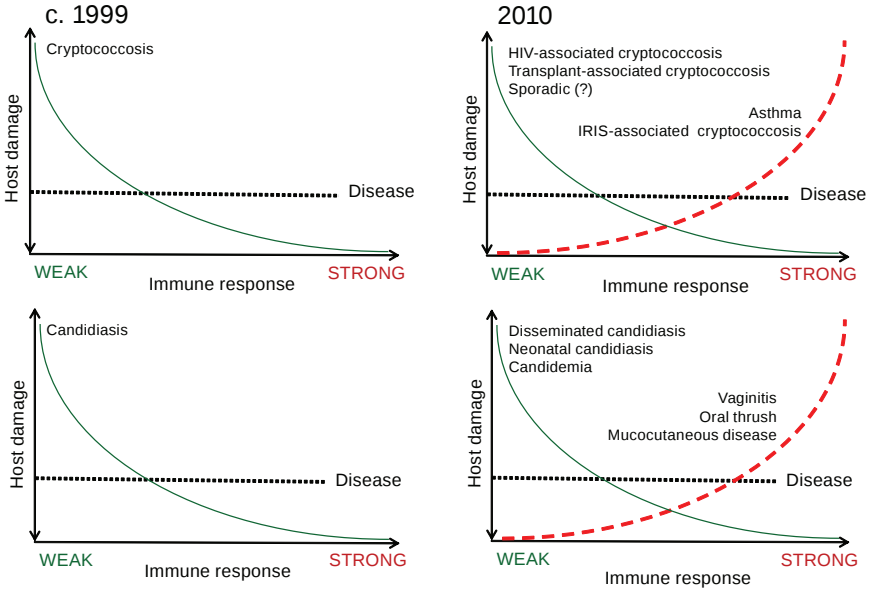


FIGURE WO-10 Damage response framework. When the framework was proposed in the late 1990s, conventional thinking was that the stronger the immune system response, the less damage to the host, as depicted in the two lefthand graphs (*Cryptococcus* spp. on the top, *Candida* spp. on the bottom). Since then, it has become clear that this is not the case and that people with very strong immune systems can also become sick, as depicted in the two righthand graphs. On the top righthand graph, notice that HIV-positive individuals with weak immune systems are at risk of HIV-associated cryptococcosis, but HIV-positive individuals with reconstituted immune systems (i.e., via antiretroviral therapy) are at risk of immune reconstitution inflammatory syndrome (IRIS)-associated cryptococcosis. On the bottom righthand graph, notice that candida vaginitis is believed to be associated with an overreactive immune system.
SOURCE: Casadevall (2010).

He noted that “we are dealing with things now that we never saw 30–40 years ago. The elimination of many viral and bacterial exposures, especially early in life, without the concomitant elimination of fungal diseases could be a factor in asthma and other atopic diseases.” Relman added, “We are not good at measuring subtle damage. If there are fundamentally important but less obvious forms of damage going on in the environment due to the emergence of fungi, we are not going to be very swift at detecting them, or insightful about understanding their implications.” Infectious propagules⁴⁹ of *C. neoformans* spp. are everywhere, Casadevall stated, and “we are all exposed to them.” Despite this high level of

⁴⁹ Spores or desiccated, encapsulated yeast cells.

exposure, Casadevall observed, *C. neoformans* infection rarely causes illness in non-immunocompromised individuals. Casadevall went on to suggest that asthma in children may be linked to an immune system that has been thrown out of immunological balance by chronic exposure to *C. neoformans*. To support this hypothesis, Casadevall pointed to two studies: Goldman et al. (2001) reported that *C. neoformans* infects the majority of immunocompetent children ages 2 and older,⁵⁰ and animal studies that demonstrated that even when asymptomatic, chronic cryptococcal infection predisposes an individual to asthma (Goldman et al., 2006).

Microbial Flora

Host immune defenses against fungal disease extend to their microbial flora. As speaker Vance Vredenburg of San Francisco State University explained, amphibians “wear their defenses on their skin” (e.g., glands produce defensive toxins). (Dr. Vredenburg’s contribution to the workshop summary report can be found in Appendix A, pages 342–355.) Indeed, Brucker et al. (2008) isolated a strain of bacteria (*Janthinobacterium lividum*) from the skin of the red-backed salamander (*Plethodon cinereus*) and demonstrated that the bacteria produced antifungal metabolites at concentrations lethal to the causative agent of amphibian chytridiomycosis (*Bd*) (Figure WO-11).

Host Behavior and Thermal Tolerance

Other host characteristics including individual or group behavior can contribute in unexpected ways to disease establishment and spread. Several participants noted that behaviors such as clustering for warmth (amphibians) or during hibernation (bats) may increase opportunities for pathogen transmission between animals. According to Casadevall, having a body temperature that exceeds the thermal tolerance of fungi⁵¹ may also be a significant host defense (Robert and Casadevall, 2009). Casadevall explained that most fungi thrive in the temperature range of 12°C to 30°C. The mammalian body temperature of 37°C, he speculated, may represent a balance between warding off fungal infection (not too cold, or too close to ambient) and keeping metabolic costs down (not too hot) (Bergman and Casadevall, 2010).

⁵⁰ Based on sera obtained from individuals who were being evaluated in an urban emergency department.

⁵¹ Robert and Casadevall (2009) found that of the 4,802 fungal strains examined (from 144 genera), most could not grow at mammalian temperatures, and that every “1°C increase in the 30°C–40°C range excluded an additional 6 percent of fungal isolates,” implying that fever could significantly increase the thermal exclusion zone. This led them to conclude that, “Mammalian endothermy and homeothermy are potent nonspecific defenses against most fungi that could have provided a strong evolutionary survival advantage against fungal diseases.” See contributed manuscripts by Casadevall in Appendix A (pages 177–188).

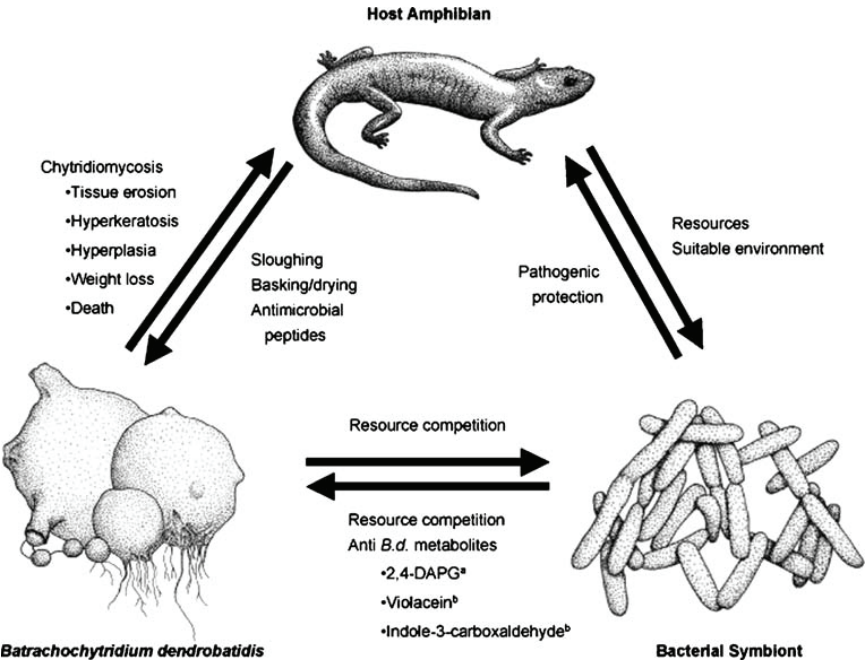


FIGURE WO-11 Microbial flora as a host defense. Components of a host’s microbial flora can be beneficial to the host by competing for resources or by secreting compounds that affect the survival of other, potentially harmful microbial components of the flora. SOURCE: With kind permission from Springer Science+Business Media: Journal of Chemical Ecology, Amphibian Chemical Defense: Antifungal Metabolites of the Microsymbiont *Janthinobacterium lividum* on the Salamander *Plethodon cinereus*, 34, 2008, 1422–1429, R.M. Brucker, Figure 1.

Pathogen Adaptation

Many fungi do not need a living host to survive. Fungi are well adapted to exploit winds and water as a means for their dispersal. Moreover, a variety of “environmental” cues trigger fungal growth, sexual and asexual reproduction, sporulation, and continued existence during adverse environmental conditions (Bahn et al., 2007; Judelson and Blanco, 2005; Kauserud et al., 2008; Kumamoto, 2008). In response to environmental stimuli, such as heat or drought, fungal organisms can become “dormant”—an inactive state during which growth and development cease but from which the organisms can be revived—or transform into forms that are resilient to heat, drought, and winds. As discussed at the meeting, the environment and environmental stimuli may also serve as a reservoir and

trigger for fungal pathogen adaptation and evolution (Casadevall, 2007; Lin and Heitman, 2006; Stukenbrock and McDonald, 2008).

Sexual reproduction in fungi typically requires the presence of two different mating types (Heitman, 2006). Two signals that regulate the sexual cycle of *C. gattii* are interactions with plants and extreme desiccation (Lin and Heitman, 2006; Xue et al., 2007). According to Heitman, evidence suggests that when only one mating type is present in an environment, *C. gattii* will adopt a “same-sex” mating strategy for reproduction (Fraser et al., 2005; Lin et al., 2005; Saul et al., 2008). This adaptability may be a widespread phenomenon, one that enables recombination, the generation of genetic diversity, and the geographic expansion of fungi (Heitman, 2006, 2009).⁵²

Same-sex mating may have contributed to the expansion of *C. gattii*’s geographical range to Vancouver Island and the U.S. Pacific Northwest (Fraser et al., 2005). Heitman also discussed how recombination between *C. gattii* lineages of the same “sex” may have resulted in a “hypervirulent” recombinant genotype associated with the outbreak. Two of the three pathogen genotypes associated with the *C. gattii* outbreak (VGIIa and VGIIc) are considered highly virulent (Byrnes et al., 2010; Fraser et al., 2005). Moreover, VGIIa is considerably more virulent than VGIIa isolates from other parts of the world (Fraser et al., 2005). Although the reason why the VGIIa and VGIIc genotypes are so virulent is unclear, there may be a link between the capacity for mating and production of spores and virulence (Byrnes et al., 2010; Fraser et al., 2005; Lin and Heitman, 2006).

Howlett explained that plant breeders consider fungal pathogens to have a “high evolutionary potential” if organisms undergo prolific sexual reproduction and produce large numbers of genetically diverse spores that then act as inoculum. This capacity leads to frequent breakdowns in a host plant’s resistance to infection by particular strains of a fungal pathogen. Howlett also explained how the agricultural environment plays a role in the breakdown of resistance.

Agricultural crops are large swaths of genetically identical plants that “exert high levels of selection pressure” on populations of fungal strains produced during sexual reproduction. Of the billions of offspring produced, the few fungal strains that can infect these “resistant” plant strains will be amplified with each subsequent disease cycle. As the frequency of virulent pathogens increases, host resistance to disease eventually breaks down. Howlett noted that this is exactly what happened with *Leptosphaeria maculans*, the causative agent of blackleg in canola. In 2000, a new cultivar⁵³ of *L. maculans* with a major resistance gene was released on the Eyre Peninsula, Australia. Within 3 years, the fungal pathogen had developed the capacity to overcome the host species’ genetic resistance resulting in yield losses of more than 90 percent (Sprague et al., 2006).

The environment can also serve as a reservoir for pathogen adaptation and

⁵² See contributed manuscript by Heitman in Appendix A (pages 226–248).

⁵³ A variety of a plant that has been created or selected intentionally and maintained through cultivation.

evolution. Fungal pathogens that are free living in the environment may acquire what Casadevall called “accidental virulence.” He noted that the soil can be an extreme environment and that soil-dwelling microbes must adapt to rapidly changing, often harsh, conditions (Casadevall and Pirofski, 2007). Traits acquired in this environment, which allow fungal species to survive predation from amoeba and other protozoan organisms, may also contribute to virulence capabilities in hosts never before encountered by fungal pathogens. Casadevall suggested that the concept of “accidental virulence” might best describe how environmentally acquired fungi can be so virulent in “new” mammalian and other host organisms (Casadevall, 2007; Casadevall and Pirofski, 2007).

EMERGING FUNGAL DISEASES OF HUMANS, ANIMALS, AND PLANTS

Several case studies of emerging fungal disease were discussed at the workshop. These case studies illustrate the many factors that influence disease emergence, the myriad direct and indirect impacts of fungal diseases on human and ecosystem health, and the challenges of detecting and responding to these infectious diseases.

Cryptococcus gattii

C. gattii already had the ability to survive in a wide range of environmental variations, but the Western North America outbreak teaches us that it may exploit hitherto unrecognized but clement environments and provide a wider exposure, and thereby, risk of infection to the human and animal populations.

—Datta et al. (2009b, p. 5)

Cryptococcus gattii (*C. gattii*) is a pathogenic, environmental fungus that emerged in humans and domestic animals on Vancouver Island, British Columbia, Canada, in 1999, causing a growing epidemic of human and animal infections and deaths. The fungus, which causes deadly infections of the lung and brain, had been previously restricted to the tropical or subtropical regions of Australia, the South Pacific, Southeast Asia, and Africa (Datta et al., 2009a,b). Since its initial recognition in 1999 as an emerging disease, the outbreak has spread from Vancouver Island to the British Columbia mainland and south into the Pacific Northwest of the United States (Datta et al., 2009b) (Figure WO-12).

According to speakers Julie Harris, of the Centers for Disease Control and Prevention, and Karen Bartlett, from the University of British Columbia, as of December 2010, this fungal pathogen has been associated with approximately 338 confirmed human infections and at least 40 deaths. (Dr. Harris’ contribution to the workshop summary report can be found in Appendix A, pages 207–225; Dr. Bartlett’s contribution to the workshop summary report can be found in

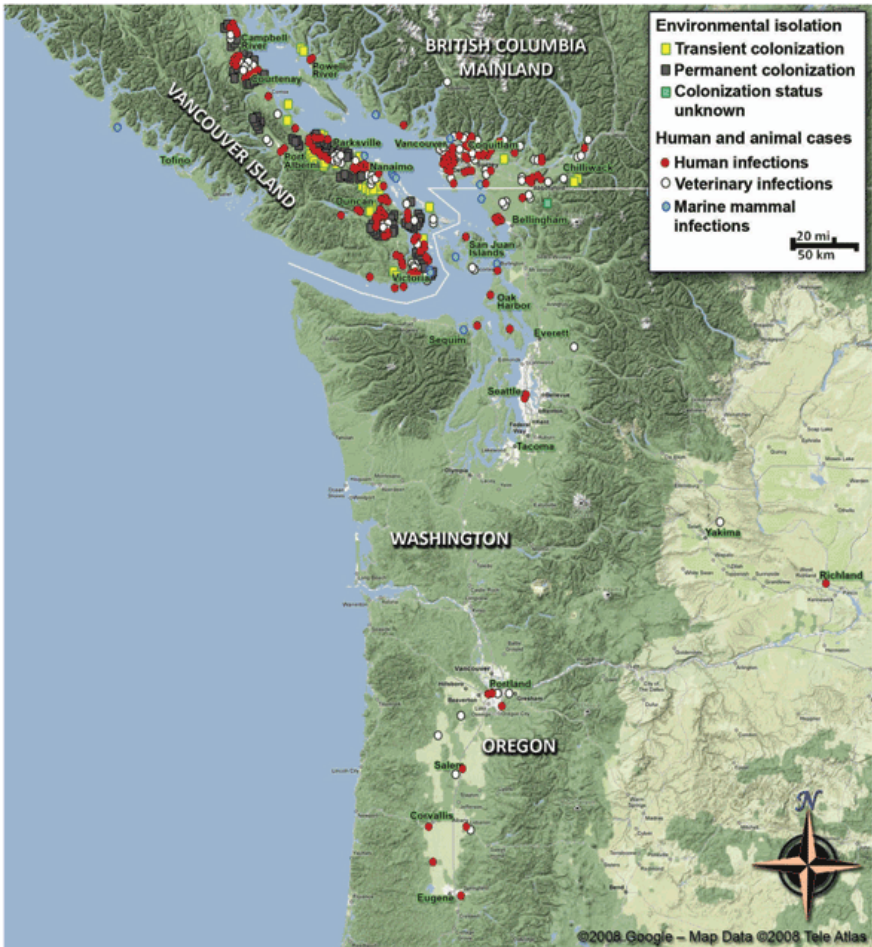


FIGURE WO-12 Map of the Pacific Northwest, comprising parts of British Columbia, Canada, and the states of Washington and Oregon in the United States, showing human and veterinary *Cryptococcus gattii* cases (including marine mammals) by place of residence or detection, and locations of environmental isolation of *C. gattii* during 1999–2008 (strain NIH444 [Seattle] or CBS7750 [San Francisco] not included). Data were collected from various state health departments and published reports referenced in the text. The map and icons have been used at a scale that shows gross geographic areas, effectively masking any personally identifiable patient locality information. Use of the map is courtesy of exclusive permission from Google Maps: ©2008 Google, map data ©2008 NAVTEQ. SOURCE: Datta et al. (2009a).

Appendix A, pages 101–116.) Investigators still do not know the origins of the current epidemic, how *C. gattii* was introduced into the Pacific Northwest, or how this invasive fungal pathogen is spreading (Datta et al., 2009a).

Phenomenology

C. gattii is a basidiomycetous yeast that colonizes tree bark, decaying wood, and nearby soil and is a cause of cryptococcosis, a potentially fatal infection in humans and animals (Galanis and MacDougall, 2010; Levitz, 1991; Lin and Heitman, 2006; MacDougall et al., 2007). Before the current outbreak in British Columbia, Canada and the Pacific Northwest, the environmental source with which *C. gattii* had been most often associated was the wood, bark, and detritus of eucalyptus trees (Levitz, 1991). More recent and widespread global surveillance has established that the fungus also colonizes other tree species (Lin and Heitman, 2006).⁵⁴

Individuals become exposed to *C. gattii* by inhaling the organism or its spores from soils or trees that have been colonized by the fungus (Lin and Heitman, 2006; Sorrell, 2001). Once inhaled, *C. gattii* can cause severe infection of the lungs and brain, including pneumonia, meningoencephalitis, and cryptococcomas. Unlike *C. neoformans*, which has become a major cause of death in HIV-infected individuals around the world, *C. gattii* also infects apparently healthy, immunocompetent individuals (Galanis and MacDougall, 2010). The disease affects a wide variety of humans and animals, but no case of transmission between animals and/or humans has ever been documented (CDC, 2010; Datta et al., 2009a).

Timely diagnosis of a *C. gattii* infection can be difficult. Patients infected with this fungal pathogen often remain asymptomatic for 6 months or more. When symptoms do present, fungal agents are not commonly considered by physicians when evaluating pulmonary disease in an otherwise healthy patient (Knox, 2010). Treating infected individuals can also be challenging because the disease tends to require prolonged antifungal therapy, sometimes with multiple drug courses (Iqbal et al., 2010; Sorrell, 2001). Some have suggested that when compared with *C. neoformans*, *C. gattii* infections tend to require more prolonged and invasive treatment (Sorrell, 2001). Harris remarked that “existing data suggest that not all cryptococcal infections are alike” and “it is not clear which factors are the most influential on the patient’s presentation—the species, subtype, host immune status, or host genetics, or some combination of factors.”⁵⁵

⁵⁴ See contributed manuscript by Bartlett in Appendix A (pages 101–116).

⁵⁵ For more information, see contributed manuscripts by Harris and Heitman in Appendix A (pages 207–225 and 226–248).

Discovery and Spread

Veterinarians and clinicians first observed cases of *C. gattii* infections in animals and humans on Vancouver Island in 1999 (Datta et al., 2009a). Until 2004, all known human cases of *C. gattii* infection in the region occurred in individuals who either resided on or visited the island, specifically its eastern coast (Bartlett et al., 2007). In 2004, cases of *C. gattii* infections emerged on the British Columbia mainland in humans and animals that had not visited Vancouver Island, suggesting an expansion of the endemic zone of the fungus (Datta et al., 2009a; MacDougall et al., 2007). Also in 2004, cases of humans infected with *C. gattii* who had not traveled to British Columbia emerged in Washington and Oregon, marking the southern expansion of the fungus into the United States (CDC, 2010; Datta et al., 2009a; MacDougall et al., 2007). The emergence and recognition of a new, more virulent strain of the fungus accompanied *C. gattii*'s expansion into Oregon (Byrnes et al., 2009, 2010). Recently, investigators found evidence that the outbreak may have also expanded into other states, as researchers have collected isolates from humans and animals in California and Idaho (Iqbal et al., 2010).

Animal sentinels were instrumental to the study of *C. gattii* emergence and spread in British Columbia (Bartlett et al., 2007). Bartlett noted that "human cases, in all cases, were preceded by veterinary cases." There are at least three to four times as many pet cases as human cases, she continued, and "it was a veterinarian that tipped us off that we had an outbreak." Harris remarked that *C. gattii* "is not a picky pathogen," infecting a wide variety of animals, including, but not limited to dogs, cats, dolphins, porpoises, elks, llamas, Bactrian camels, alpacas, horses, and sheep.

The exact means by which *C. gattii* spread from Vancouver Island to the British Columbia mainland, and south to the United States, remains unknown. Researchers suspect that a number of factors may be responsible, including human-mediated dispersal. A 2007 sampling study in British Columbia found *C. gattii* in areas of its endemic zone that were subject to high foot and vehicle traffic. These observations, combined with the finding of positive fungal samples on peoples' shoes and in the wheel wells of vehicles that had been driven in fungal endemic regions, support the contention that dispersal may be partially anthropogenic (Kidd et al., 2007). Forestry activities could also facilitate *C. gattii* dispersal by aerosolizing fungus particles during tree cutting and/or mechanically "seeding" the fungus during the transfer of cut tree products, such as mulch, to new areas (Kidd et al., 2007). In addition to these human-mediated dispersal methods, Kidd and colleagues (2007) suggested that birds and animals might also play a role by passively transporting the fungus during migration.

As reviewed by Bartlett, environmental sampling of endemic areas has helped to describe how *C. gattii* is distributed in the environment and how easily it might spread. Sampling revealed high levels of *C. gattii* in the soil as well as its presence in the air, freshwater, saltwater, trees, and even dead wood, such as

fence posts, Bartlett remarked. Sampling results have also illustrated the effects of forestry activities on the abundance of *C. gattii* in the air (measured in colony-forming units,⁵⁶ or CFUs). According to Bartlett, air samples taken in endemic areas, where trees were being removed, revealed a baseline concentration on the order of 100 CFU/m³, compared to 10,000 CFU/m³ during tree chainsawing and wood chipping in the same area (Kidd et al., 2007). Once it is in the air, Bartlett observed “the organism can travel 10 kilometers, easily, probably further than that.” The organism is also resilient: Bartlett noted that she can still isolate viable propagules from sawdust samples taken in 2001.

Origins of the Outbreak in the Pacific Northwest

The origins of the Pacific Northwest outbreak of *C. gattii* remain a mystery (Datta et al., 2009a). Some investigators have suggested that the fungus was introduced through the importation of contaminated trees, shoes, wooden pallets, or shipping crates (Kidd et al., 2007). Supporting this hypothesis is the finding that the VGIIb minor subtype found in British Columbia and the Pacific Northwest is similar and may be related to VGIIb strains found in Australia (Byrnes et al., 2010). Another origin hypothesis suggests that the VGIIa subtype has existed in the Pacific Northwest for some time. The latter hypothesis is supported by a case reported in 1971 of a patient in Seattle, Washington, who was infected with a VGIIa strain of *C. gattii* similar to the strain in the current outbreak (Byrnes et al., 2010; Datta et al., 2009a). However *C. gattii* was introduced, it is clear that the fungus is now established in the region and appears to be evolving into new, more virulent strains, as evidenced by the newly discovered and highly virulent VGIIc strain (Byrnes et al., 2010; Knox, 2010).

Perhaps more interesting than the question of how *C. gattii* was introduced to British Columbia and the Pacific Northwest is why it has now colonized the region. Bartlett emphasized that the fungus previously was endemic only in areas with tropical or subtropical climates—never in a temperate rainforest. Researchers have speculated that global warming may play a role, with the temperature in the region having increased enough for the fungus to become established (Bartlett et al., 2007; Kidd et al., 2004). Indeed, between 1998 and 2004, British Columbia experienced six consecutive seasons of above-average temperatures, with increases of more than 3°C in some seasons (Kidd et al., 2004).

Another possible explanation for *C. gattii*’s establishment in British Columbia and the U.S. Pacific Northwest is that the organism itself has adapted such that it can now successfully colonize a “novel” environment. Investigators have found environmental isolates of *C. gattii* in trees that had never previously been found to harbor the fungus, such as the Douglas fir and Western hemlock (Datta

⁵⁶ CFUs are a standard unit of measurement for environmental sampling. Colonies reflect the number of “viable” organisms (i.e., organism capable of forming colonies when provided with nutritional elements necessary for growth).

et al., 2009a). Bartlett remarked that the environmental sampling data may help to define *C. gattii*'s ecological niche in British Columbia. The distribution of *C. gattii* in the environment, thus far, appears heterogeneous, with colonization levels differing significantly in regions such as the west and east coasts of Vancouver Island, which, observed Bartlett, are "dramatically different in terms of rainfall, soil type and vegetation" (Figure WO-13).

Further research into the reasons why *C. gattii* emerged in the temperate rainforest of the Pacific Northwest is needed because it could help researchers predict the fungus's future spread and further the scientific community's understanding of how environmental pathogens establish themselves in new environmental niches.

Molecular Epidemiology, Virulence, and Drug Resistance

Heitman explained that *C. gattii* spans four genetically isolated species groups: VGI, VGII, VGIII, and VGIV. Examination of the molecular genotypes of fungal isolates from infected patients reveals that nearly all of the observed infections in British Columbia and the U.S. Pacific Northwest have been caused

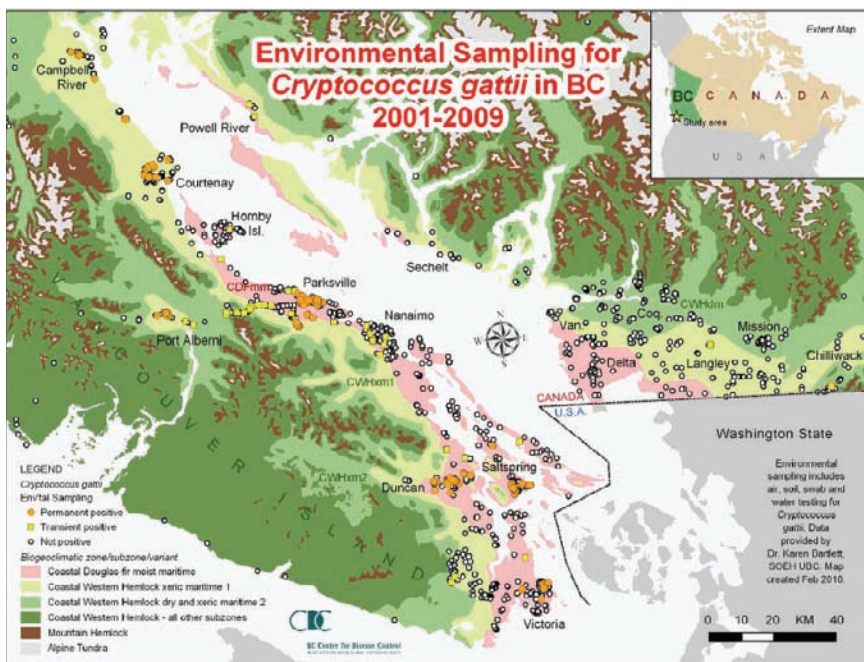


FIGURE WO-13 Environmental sampling for *Cryptococcus gattii* in British Columbia (2001–2009). Colonization levels of *C. gattii* reflect a heterogeneous distribution of *C. gattii* in the environment. Biogeoclimatic zones are also indicated.

SOURCE: Bartlett (2010).

by one molecular subtype of *C. gattii*—the VGII type (Byrnes et al., 2009). In other regions of the world where *C. gattii* is endemic, two other molecular subtypes predominate—VGI and VGIII (Byrnes et al., 2009; Kidd et al., 2004). The VGII genotype is further subdivided into three subtypes: the majority genotype VGIIa, which is unique to the Pacific Northwest region and not found in other endemic regions; the less common VGIIb genotype; and the VGIIc subtype, which has appeared in Oregon within the past several years (Byrnes et al., 2010; Kidd et al., 2004).

Some researchers have suggested that the predominant VGIIa and the newly discovered VGIIc *C. gattii* subtypes are more virulent than strains found in other endemic countries such as Australia (Byrnes et al., 2010). This is supported by the high rate of *C. gattii* infections in the current outbreak, which, at 25.1 cases/million people on Vancouver Island, is among the highest in the world (Galanis and MacDougall, 2010). Bartlett cautioned, however, that the high rate of *C. gattii* infection could be a result of increased surveillance or exposure, not increased virulence.

Recently, researchers have compared the drug susceptibility of the three VGII subtypes found in British Columbia and the Pacific Northwest to the more common VGI and VGIII genotypes. The VGIIc strain was found to be significantly more drug resistant to nearly all of the tested antifungal compounds (voriconazole, fluconazole, flucytosine, and amphotericin B) than the VGI or VGIII genotypes. The VGIIa and VGIIb strains were also observed to be more resistant to some antifungal drugs (fluconazole, flucytosine, and amphotericin B for VGIIa; fluconazole for VGIIb) than the VGI and VGIII strains, though their levels of resistance were lower than those of the VGIIc strain (Iqbal et al., 2010).

White-Nose Syndrome in Bats

Last year we estimate that we found between 10,000 and 20,000 dead bats on the cave floor . . . and to be honest the mortality is so disturbing. . . . We just can't crawl through so many piles of dead bats.

—Scott Darling, Vermont Fish and Wildlife Department
(Buchen, 2010, p. 144)

Since the winter of 2006, a mysterious and previously unknown disease—bat white-nose syndrome (WNS)—has decimated hibernating bat populations in the eastern and central United States. Named after the visually distinctive white fungus—*Geomyces destructans*—that grows on the muzzles, ears, and wings of affected bats, the disease has spread rapidly across the United States and Canada. Since it was first discovered, WNS has killed more than 1 million bats in the United States, with some hibernation sites (hibernacula) losing 90–100 percent of their bat populations (Figure WO-14) (FWS, 2011).

The population declines have been so rapid and dramatic that bat biologists



FIGURE WO-14 Signs of bat white-nose syndrome (WNS). (A) Mortality caused by WNS. (B) *Geomyces destructans* fungus, forming the visually distinctive white growth on the muzzle, ears, and wings of an infected bat.

SOURCE: Photos provided courtesy of Alan C. Hicks, New York State Department of Environmental Conservation.

at the U.S. Fish and Wildlife Service fear the extinction of entire New World⁵⁷ bat populations in the United States and Canada (FWS, 2011).

Such extinctions could have devastating ecological and economic consequences. Bats play a critical role in plant pollination, seed dissemination, and the control of flying insects, including mosquitoes, moths, beetles, and other night-flying insect populations (Blehert et al., 2009; Boyles et al., 2011). Large-scale declines or complete disappearances of bat populations could result in reduced plant pollination (which is already under siege by colony collapse disorder in honeybees), significant increases in “nuisance” insect populations, and increased insect damage to agricultural and forestry resources (Blehert et al., 2009; Boyles et al., 2011; FWS, 2011). Because bats have very low reproductive rates—producing one pup a year, sometimes two, in a single litter—WNS is predicted to have long-lasting effects (Barclay et al., 2004; FWS, 2011). The value of bats to the agricultural industry has been estimated to be roughly \$22.9 billion/year, with a range per year of \$3.7 billion to \$53 billion (Boyles et al., 2011).

⁵⁷ Refers to the Western Hemisphere; in a biological context, New World species are those from the Nearctic Neotropic ecological zones, versus Old World species from the Palearctic and Afrotropic ecological zones.

Phenomenology

Geomyces destructans hyphae⁵⁸ and conidia⁵⁹ invade the hair follicles and sebaceous and sweat glands of bats hibernating in caves and mines with seasonal temperature ranges between 2°C and 14°C (Blehert et al., 2009). The skin of affected bats does not typically show signs of inflammation or an immune response at the site of fungal invasion (Meteyer et al., 2009). Hibernating bats infected with WNS often have severely depleted fat reserves, which are critical for successful hibernation (Blehert et al., 2009).⁶⁰

Researchers have not yet confirmed whether *Geomyces destructans* is the primary pathogen that causes WNS and the eventual death of affected bats, or if it is an opportunistic infection that invades animals already immunocompromised by some other, yet to be defined pathogen (Puechmaille et al., 2010). How WNS kills bats is also unclear. Infection by the pathogen may irritate the animals, rousing them from hibernation, and causing them to deplete their fat reserves to such an extent that they are unable to survive through the winter (Blehert et al., 2009; Cryan et al., 2010; FWS, 2011). Speaker David Blehert of the National Wildlife Health Center at the U.S. Geological Survey presented recent research suggesting that the fungus causes damage to the wing epidermis and skin structures that help protect against water loss, causing bats to lose too much water to survive their winter hibernation (Cryan et al., 2010). (Dr. Blehert's contribution to the workshop summary report can be found in Appendix A, pages 167–176.)

Discovery and Spread

WNS was first documented in February 2006 in a single cave near Albany, New York (FWS, 2011). Since then, WNS has spread rapidly across the United States and Canada, killing more than a million bats of six different species, making it the “worst wildlife health crisis in memory” (FWS, 2011). WNS is thought to spread primarily through bat-to-bat contact. However, human cavers and tourists may also be contributing to the spread of this pathogen by inadvertently transporting spores of *Geomyces destructans* from cave to cave on their clothing and equipment (Frick et al., 2010; FWS, 2011).

During routine hibernacula surveys in 2006–2007, biologists with the New York Department of Environmental Conservation discovered bats exhibiting signs of WNS in five caves, all within a 15-km radius of what is now recognized as the likely “index” site near Albany, New York, recounted Blehert. The following winter (2007–2008), researchers reported the discovery of WNS at 33 sites across

⁵⁸ Slender tubes that develop from germinated spores and form the structural parts of the body of a fungus. A large mass of hyphae is known as a mycelium, which is the growing form of most fungi.

⁵⁹ Asexually produced fungal spore. Most conidia are dispersed by the wind and can endure extremes of cold, heat, and dryness. When conditions are favorable, they germinate and grow into hyphae.

⁶⁰ See contributed manuscript by Blehert in Appendix A (pages 167–176).

Connecticut, Massachusetts, New York, and Vermont—all within a 210 km radius of the index site (Blehert et al., 2009). By the end of the 2010–2011 hibernation season, the disease had been confirmed in bats in 18 U.S. states (FWS, 2011). The disease has also spread north into Ontario, Quebec, New Brunswick, and Nova Scotia in Canada (FWS, 2011) (Figure WO-15).

Blehert remarked that the magnitude of the mortality events being observed is not only unprecedented among U.S. bat species but also among the 1,100-plus bat species worldwide. Indeed, a recent modeling study predicted that for one of the most common bat species in North America—the little brown bat (*Myotis lucifugus*)—there is a 99 percent chance of regional extinction within the next 16 years as a result of mortality from white-nose syndrome (Frick et al., 2010).

Affected New World bat species include the big brown bat (*Eptesicus fuscus*), Eastern small-footed bat (*Myotis leibii*), little brown bat (*M. lucifugus*), Northern long-eared bat (*M. septentrionalis*), endangered Indiana bat (*M. sodalis*), and tricolored bat (*Perimyotis subflavus*) (Figure WO-16). Although disease pathology has not been confirmed, DNA from *G. destructans* has been detected

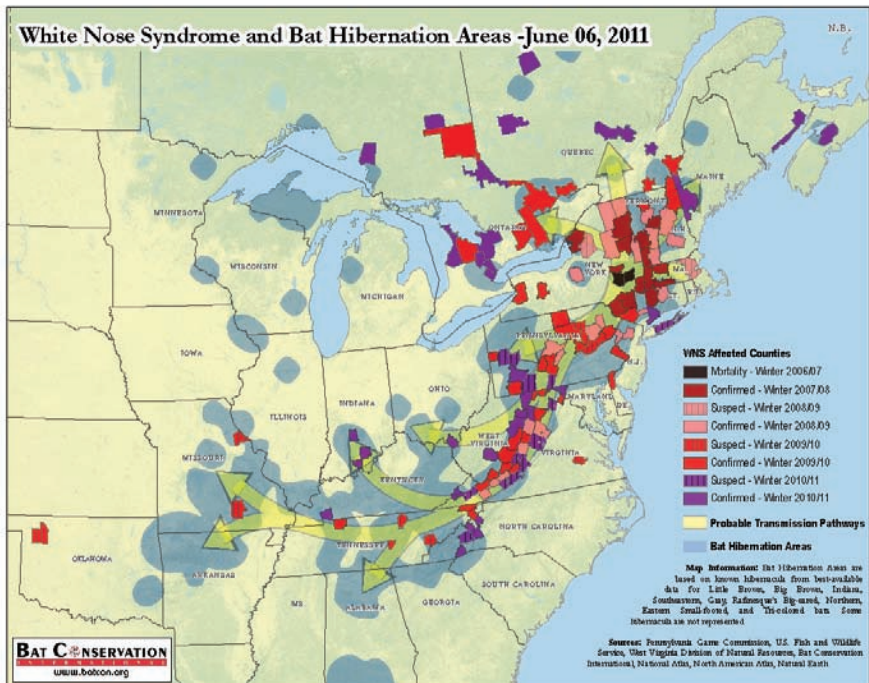


FIGURE WO-15 Spread of bat white-nose syndrome (WNS) in North America as of June 6, 2011.

SOURCE: Figure courtesy of Bat Conservation International, www.batcon.org.



FIGURE WO-16 Species affected by bat white-nose syndrome (WNS).

SOURCE: Merlin D. Tuttle, Bat Conservation International, www.batcon.org.

on skin samples from the endangered grey bat (*M. grisescens*), the southeastern bat (*M. austroriparius*) and the cave bat (*M. velifer*) (FWS, 2011). Investigators are concerned that the endangered Virginia big-eared bat (*Corynorhinus townsendii virginianus*) may also be “at risk.” Although the fungus has been detected on other bat species that share their hibernacula, there has yet to be confirmed case of an infected animal (FWS, 2011).

Investigators are concerned that WNS could eventually spread to infect all 25 of the hibernating bat species native to the United States, threatening more than 50 percent of the native U.S. bat populations (Bat Conservation International, 2010). In late May 2010, FWS officials reported that a live bat from Oklahoma was PCR-positive for *G. destructans* DNA. This finding alarmed many, because the infected species, the cave bat *M. velifer*, frequently shares hibernacula with other bat species with migratory ranges that extend across the western United States into Mexico, increasing the potential for further spread of the disease to the west and south (Bat Conservation International, 2010; Oklahoma Department of Wildlife Conservation and FWS, 2011). This finding, however, was not confirmed by fungal culture or histopathology; and, to date, WNS has not been confirmed in states west of the Mississippi River.

Bat researchers and human cavers may also be contributing to the rapid spread of this pathogen within and across states. Blehert noted that the index site for WNS, Howes Cave, is connected to Howe Caverns, a commercial tourist cave that entertains up to a quarter-million visitors per year. Although there are no data supporting (or refuting) the hypothesis that humans are serving as transmission

“vectors,” Blehert noted that the U.S. Fish and Wildlife Service recommends that all people who enter caves (e.g., researchers and speleologists) employ decontamination protocols for potentially contaminated clothes and equipment.

Origins

The origins of WNS and its relationship to *G. destructans* remain unknown (Qaummen, 2010). However, recent evidence from Europe and characterization of the newly described pathogen may provide clues. In early 2010, researchers published several reports confirming that a number of bats (all *Myotis* spp.) in France, Hungary, Germany, and Switzerland, while infected with *G. destructans*, remained healthy (Puechmaille et al., 2010; Wibbelt et al., 2010). Speaker Gudrun Wibbelt, of the Leibniz Institute for Zoo and Wildlife Research, reported that not all of the European hibernacula surveyed have infected bats, and of those that do, often only a small number of bats within the colony are infected. (Dr. Wibbelt’s contribution to the workshop summary report can be found in Appendix A, pages 368–403.) Researchers have now confirmed the presence of *G. destructans* on 8 species of Old World bats in 12 countries in Europe (Puechmaille et al., 2011).⁶¹ In addition, photographic evidence suggests that the fungus was present on bats in Europe at least as early as the 1980s (Wibbelt et al., 2010). These findings, which have important implications for future WNS research, could help explain the origins of the disease and may also provide clues for understanding the mechanisms of the infection.

One possible interpretation of these data is that *G. destructans* may have originated in Europe and that Old World bats and this pathogen may have co-evolved (Puechmaille et al., 2011; Wibbelt et al., 2010). This hypothesis is supported anecdotally by reports from routine winter bat surveys in Europe from the past 30 years. The surveys occasionally noted the presence of a white fungus similar in appearance to *G. destructans* on otherwise healthy bats, Wibbelt noted. If European bats have coevolved with the fungus, they might be able to muster a sufficient immune response to control and survive infection by *G. destructans* (Puechmaille et al., 2010). Some have proposed the possibility that the microbial flora of bat skin or other abiotic surfaces in European hibernacula “may have also coevolved to incorporate *G. destructans* as a non pathogenic component of the microbial community” (Wibbelt et al., 2010). Wibbelt also reported the possibility that Old World bats may only be colonized in a superficial fashion on the outer epidermis without any invasion into deeper tissues.

A second possible interpretation of the discovery of healthy European bats infected with *G. destructans* is that disease transmission in Old World bat populations may be affected for some biological or behavioral reason (Puechmaille et al., 2010, 2011; Wibbelt et al., 2010). European bats tend to hibernate in

⁶¹ See contributed manuscripts by Wibbelt in Appendix A (pages 368–403).

relatively small groups (rarely more than 100 individuals per cluster), Wibbelt explained. This might make it more difficult for the disease to spread. In the United States, bats hibernate in groups that can reach into the hundreds of thousands (Wibbelt et al., 2010).

Additional explanations for why European bats infected with *G. destructans* do not succumb to WNS include the possibility that the fungal strain in the United States is more virulent than the strain in Europe or that *G. destructans* is not the primary cause of death in WNS. However, Blehert did note that his team's and others' diagnostic investigations of infected New World bats had ruled out toxins, parasites, and known viral and bacterial pathogens as associated with WNS (Blehert et al., 2009; Chaturvedi et al., 2010; Gargas et al., 2009). Wibbelt observed that *G. destructans* isolates from North America and Europe appear identical in morphology and in the sequence of two genes (ITS and SSU) commonly used as a marker to distinguish between different species (Puechmaille et al., 2011). Further research will be necessary to determine the true cause of differences between North American and European bats infected with *G. destructans*.

Amphibian Chytridiomycosis

The effect of chytridiomycosis on amphibians has been described as the greatest loss of vertebrate biodiversity attributable to disease in recorded history.

—Vredenburg et al. (2010, p. 9689)

Amphibians are currently the most threatened class of vertebrates on the planet (Stuart et al., 2004). Researchers classify approximately one third of the more than 6,500 known amphibian species as threatened and more than 40 percent of species have experienced population declines in recent decades (Lips et al., 2006; Lötters et al., 2010; Stuart et al., 2004). The proximate cause of many of these declines is a recently described disease associated with the fungus, *Batrachochytrium dendrobatidis* (*Bd*), which infects more than 350 species of frogs, toads, and salamanders on every continent except Antarctica (Fisher et al., 2009) (Figure WO-17). In susceptible animals, *Bd* infection causes the deadly disease chytridiomycosis, which has been implicated as the catalyst for the global decline of more than 200 amphibian species, including local extinctions of several species in the wild (Fisher et al., 2009; Kilpatrick et al., 2009; Lips et al., 2006; Lötters et al., 2010; Schloegel et al., 2006; Skerratt et al., 2007).

Until effective control measures are in place, investigators expect *Bd* to continue to threaten more species with extinction, making the fungus a candidate for the most destructive emergent, infectious epizootic disease ever recorded (Fisher, 2008). Despite its widespread impact, little is known about *Bd*'s origins, how it has spread across the globe, the specific mechanism by which it causes death, and why it is so devastating to some amphibian species while others are appar-

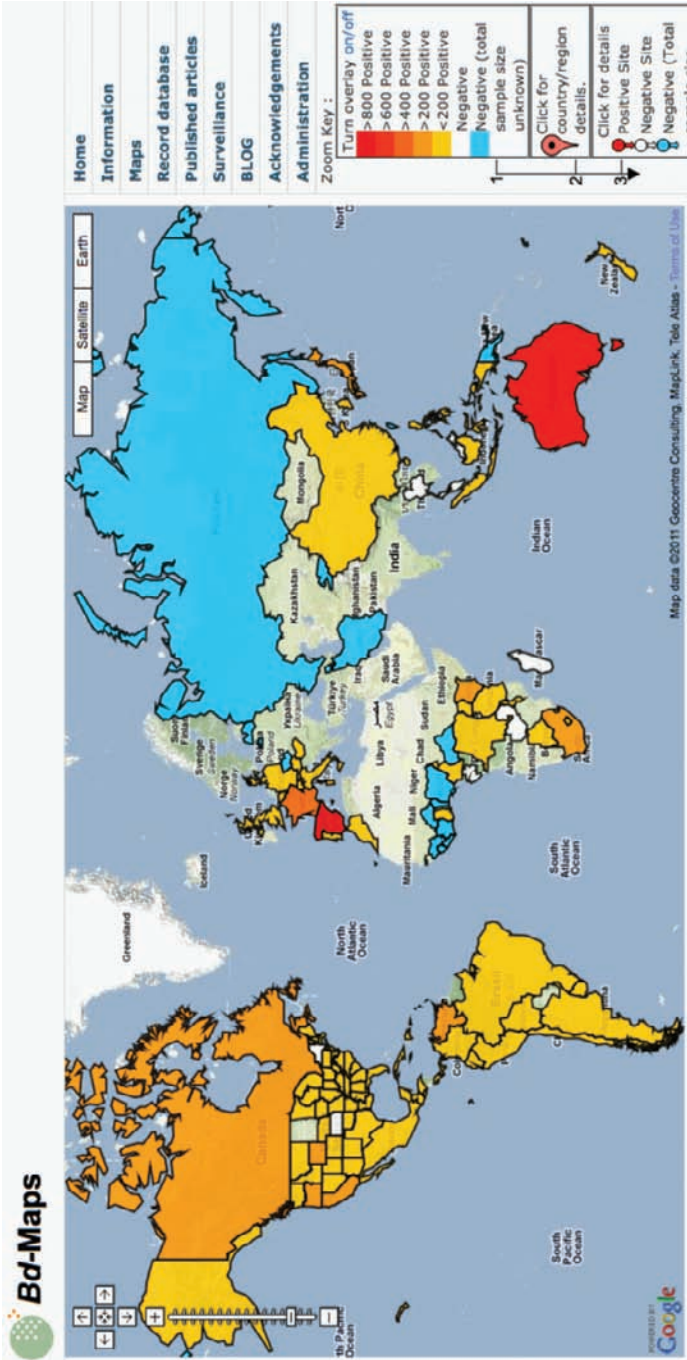


FIGURE WO-17 Global distribution of *Bd*.
SOURCE: The Global *Bd*-Mapping Project, <http://www.bd-maps.net/>.

ently able to control infections without significant morbidity or to resist infection entirely (Rosenblum et al., 2009).

Phenomenology

B. dendrobatidis is an aquatic chytrid fungus—an early diverging class of fungi—that infects keratinized epidermal cells of amphibians, causing rapidly progressing and deadly chytridiomycosis in susceptible species (Fisher et al., 2009). First identified in 1998 and characterized in 1999, *Bd* is unique among other chytrids (Berger et al., 1998; James et al., 2006; Longcore et al., 1999). It is one of only two known chytrid fungi to parasitize vertebrates and the only known species to infect the keratinized skin of *living* amphibians (Berger et al., 1998; Fisher et al., 2009). *Bd*'s asexual spore is a “free living” and motile zoospore, possessing a single flagellum that allows the spore to travel small distances (usually less than 2 cm) and thrive in aquatic habitats such as streams and ponds (Fisher et al., 2009; Kilpatrick et al., 2009; Kriger and Hero, 2007; Rosenblum et al., 2010).

According to speaker Vance Vredenburg, from San Francisco State University, investigators believe that amphibians become infected with *Bd* through both casual contacts with zoospores in the water as well as through direct animal-to-animal transmission. Once infected, the susceptible animals carry *Bd* for 24 to 220 days before they succumb to chytridiomycosis (Lips et al., 2006). Vredenburg remarked that the susceptibility to *Bd* colonization and subsequent development of chytridiomycosis varies widely across species, populations, and individuals. Indeed, laboratory experiments have found mortality rates from 0 to 100 percent, depending on temperature, species, and age of the infected animals (Berger et al., 2005; Daszak et al., 2004; Kilpatrick et al., 2009; Lamirande and Nichols, 2002; Woodhams et al., 2003).

Many of the most notable and rapid declines in amphibia have occurred among those populations living at high altitudes in mountainous regions, leading some to associate outbreaks of fatal chytridiomycosis with cooler climates and high altitudes (see Fisher et al., 2009). Several amphibian species that live near sea level also have experienced notable population declines from this fungal disease, however, suggesting that this association may be an “oversimplification of a complex host–pathogen relationship” (Fisher et al., 2009). The preponderance of the evidence supports the observation that *Bd* does prefer cooler temperatures, growing and reproducing between 4°C and 25°C (Berger et al., 2004; Drew et al., 2006; Kilpatrick et al., 2009; Kriger and Hero, 2007). Indeed, the available evidence suggests that the virulence of *Bd* is inversely related to temperature, perhaps in a species-dependent manner (Fisher et al., 2009; Walker et al., 2010).

The specific mechanisms by which some species suffer rapid declines when *Bd* is introduced while others are able to tolerate varying levels of infection without the development of disease—or even resist infection entirely—remain



FIGURE WO-18 A chytridiomycosis outbreak in southern mountain yellow-legged frogs (*Rana muscosa*), Sixty Lake Basin, Kings Canyon National Park, CA, USA.
SOURCE: Photo kindly provided by V. Vredenburg (August 15, 2006).

unknown. Nevertheless, chytridiomycosis apparently results from the complex interplay of pathogen, host, and environmental factors (Briggs et al., 2010; Rosenblum et al., 2010; Vredenburg et al., 2010; Walker et al., 2010). Recounting his investigations of disease dynamics in multiple populations of *Bd*-infected amphibians (*Rana muscosa* and *Rana sierrae*) in Sequoia and Kings Canyon National Parks in the Californian Sierra Nevada, Vredenburg noted that even populations of the same species can have “very different outcomes of infection, depending on where you are in the Sierra Nevada.”⁶²

Although numerous studies have established that *Bd* is the proximate cause of the observed declines in amphibian populations around the world, the specific ways by which chytridiomycosis causes death remains a mystery (Figure WO-18).

In susceptible animals, chytridiomycosis causes a thickening of the skin (hyperkeratosis), abnormal proliferation of epidermal cells (hyperplasia), and sometimes increased skin shedding (Berger et al., 1998, 2005). Only rarely do infected animals have visible skin lesions or other pathologies generally associ-

⁶² See contributed manuscript by Vredenburg in Appendix A (pages 342–355).

ated with lethal infections. *Bd* may disrupt the normal regulatory functions of amphibian skin, causing osmotic imbalances, electrolyte depletion, and ultimately death (Rosenblum et al., 2010). Although some investigators have suggested that *Bd* might release lethal toxins, no specific toxin has been identified (Fisher et al., 2009; Rosenblum et al., 2010). As discussed by Vredenburg, investigators are also exploring the possibility that other microbial components of the amphibian skin microbiome can contribute to disease mitigation (Harris et al., 2009).

Is Bd a Newly Emergent or Previously Endemic Pathogen?

One of the many unresolved debates among scientists who study this disease is whether *Bd* is a newly emerging pathogen that recently spread across the globe (known as the novel pathogen hypothesis) or if it has existed for some time as a commensal or symbiont of amphibians, and only recently became more virulent as a result of environmental changes that altered its relationship with its hosts (the endemic pathogen hypothesis) (Fisher and Garner, 2007; Fisher et al., 2009; Rachowicz et al., 2005; Rosenblum et al., 2009, 2010).

What is known is that *Bd* has existed at a low prevalence in some populations of amphibians since at least the 1930s, but massive species declines were not reported until recently (Weldon et al., 2004). A number of studies established significant associations between *Bd*, declines in amphibian populations, and global warming (Fisher et al., 2009; Pounds et al., 2006). Fisher remarked that more research is needed to illuminate the specific mechanisms through which climate change or other environmental factors might influence the host–pathogen dynamic between *Bd* and certain species of amphibians (Fisher et al., 2009). The preponderance of the evidence generated to date supports the novel pathogen hypothesis—*Bd* appears to be a newly emergent disease (Fisher et al., 2009; James et al., 2009; Rosenblum et al., 2009, 2010).

The most compelling evidence in support of the novel pathogen hypothesis comes from recent studies mapping the genome of *Bd* isolates from around the world (Rosenblum et al., 2009). All global diversity of *Bd* can be explained by a single ancestral diploid strain that subsequently spread across the world, diversifying through mitotic and meiotic recombination (James et al., 2009; Morgan et al., 2007). According to Fisher, investigators still do not know the origin of this single diploid strain; however, all known *Bd* strains most likely came from a small, genetically “bottlenecked” ancestral population (James et al., 2009). These data provide strong, inferential evidence in support of the theory that *Bd* is a recently emergent fungal pathogen that rapidly expanded its geographic range across the globe in the first half of the 20th century (Fisher et al., 2009; Rosenblum et al., 2009).

Origins and Spread

Despite its widespread “footprint,” little is known about *Bd*’s origins and how it has spread across the planet (Fisher et al., 2009; Kilpatrick et al., 2009; Rosenblum et al., 2009, 2010). *Bd* clearly is widely distributed, but its distribution is not homogeneous (Fisher et al., 2009). Indeed, a number of areas around the world—most notably Madagascar—have a rich diversity of amphibian species, but *Bd* has not spread there yet (Fisher et al., 2009; Weldon et al., 2008).

Skin samples from the African clawed frog (*Xenopus laevis*) collected in 1938 on the Western Cape of South Africa provide the earliest evidence of *Bd*-associated amphibian skin infections (Weldon et al., 2004). As noted by Weldon, *X. laevis* is able to asymptotically carry the fungus without developing chytridiomycosis and has not experienced any population declines as a result of *Bd* infections. In the early 20th century (between the 1930s and 1960s), the frogs were globally marketed and used in human pregnancy assays (Weldon et al., 2004). *X. laevis* is also widely used as a model organism in developmental biology because its metamorphosis from zygote to tadpole can be easily observed in the laboratory (Weldon et al., 2004). The global trade in African clawed frogs has led investigators to suggest that *Bd* originated in Africa and that *X. laevis* served as the natural reservoir host for this fungal pathogen (Weldon et al., 2007). The fungus then spread to new hosts and environmental niches as a result of the human-mediated movement of amphibians, globally and locally (Weldon et al., 2004).

Some studies have cast doubt on the hypothesis that the fungus originated in Africa (Fisher et al., 2009; Rosenblum et al., 2009, 2010). As noted by Fisher, studies of the genetic diversity of *Bd* in different species found that fungal isolates from North American bullfrogs (*Rana catesbeiana*), another species that is widely traded and is able to carry *Bd* without any associated morbidity, are significantly more genetically diverse than isolates from African clawed frogs (James et al., 2009). This is contrary to what researchers would expect if African clawed frogs were the original reservoir for *Bd* and suggests that the fungus origins may be outside of Africa (Fisher et al., 2009; Rosenblum et al., 2010). Fisher observed that genomic sequencing of global *Bd* isolates reveals that “what we have been calling *Bd* actually consists of at least three highly divergent lineages.”

A number of theories have been put forward to explain *Bd*’s spread around the globe (Fisher and Garner, 2007). As Weldon observed, many believe the spread was at least partially human-mediated, the result of international trading and transportation of amphibians that were infected with the fungus, but did not show signs of obvious illness. Anthropogenic spread through the amphibian trade, however, cannot explain how the fungus was introduced into and spread across environments where there has been minimal human activity.

***Phytophthora ramorum* in Europe and North America**

*In the USA, the economic impact of losses due to *P. ramorum* is estimated to be in the tens of millions of dollars due to the direct loss of nursery and ornamental crops, the decrease of property values due to dead/dying trees, the cost of monitoring, tracking, and eradicating the disease, the societal impact through loss of recreational value and cultural value, and the ecological impact through loss of food resources for native fauna.*

—Grünwald et al. (2008, p. 2)

The first reports of sudden oak death occurred in California forests in 1994–1995 (Rizzo and Garbelotto, 2003). While the origins of the associated pathogen, *Phytophthora ramorum* (*P. ramorum*), remain unknown, investigators believe the “source” of the subsequent epidemic in California was an infected ornamental *Rhododendron* plant(s) (Kliejunas, 2010; Mascheretti et al., 2009). Now known to cause disease (commonly known as ramorum blight) in more than 100 plant species, *P. ramorum* has also emerged as a novel plant pathogen in the United Kingdom and Europe (Grünwald et al., 2008). Collectively, these diseases have led to the rapid decline of oak forests on the west coast of the United States and have led to widespread disease in trees and woody ornamental plants throughout the United Kingdom and Europe (Grünwald et al., 2008).

P. ramorum is just one of the many invasive tree diseases that were introduced into the forests of North America in the 20th century. As in the case of chestnut blight and Dutch elm disease, the transportation and trade of plants and plant materials contributed to the movement of this pathogen across oceans and continents, as well as between suburban and forest ecosystems (Brasier and Webber, 2010; Goss et al., 2009; IOM, 2010). As reviewed by speaker Rizzo, once *P. ramorum* is established in the landscape, treatment options are extremely limited. The discovery in 2009 that *P. ramorum* was reproducing in Japanese larch trees in the United Kingdom led to the immediate clear cutting of 4 million larch trees—more than 10,000 acres of forest—in a heroic effort to slow the spread of the disease (Hardman, 2011).

Phenomenology

P. ramorum is a fungus-like oomycete⁶³ or “water mold” that thrives in the cool, wet climate of California coastal forests (Kliejunas, 2010). In contrast to most species of *Phytophthora*, *P. ramorum* exhibits a remarkably broad

⁶³ As noted by speaker Rizzo, *Phytophthora* spp. is not a “true fungus.” It is an oomycete or “water mold” that belongs to the Kingdom Stramenopila (a major eukaryotic group that includes diatoms and brown algae, and is distinct from plants, fungi, and animals). Like fungi, oomycetes “exhibit filamentous growth, produce sexual and asexual spores, and can feed on decaying matter or be obligate parasites of plants” (Kliejunas, 2010).

host range, infecting a variety of tree and non-tree species—ranging from hardwood and conifer trees, to shrubs and leafy plants⁶⁴ (Brasier and Webber, 2010; Grünwald et al., 2008). *P. ramorum* colonizes the leaves of many plants and the inner bark and sapwood of trees. The fungus can also survive in a dormant state in decaying matter, such as leaf litter on the forest floor, and in the soil (Parke and Lucas, 2008). Rizzo remarked that within forest ecosystems in California, “once we started looking, we started finding it on just about every plant we looked at, ranging from ferns to redwood trees.”

Infection occurs when spores and zoospores—dispersed by winds or water—come into contact with susceptible plants. Moisture is not only essential for the production of infectious propagules, but free water on plants—from fog, dew, or rainfall—enhances infection and dispersal (Kliejunas, 2010; Rizzo and Garbelotto, 2003). Indeed, monitoring streams that are “baited” with *Rhododendron* leaves, Rizzo said, has been an effective method for early detection of this pathogen in forest ecosystems. Disease manifests differently depending on the plant species and the part of the host plant that is infected (Grünwald et al., 2008) (Figure WO-19 and Table WO-2). Bleeding lesions and stem cankers develop in forest trees, followed by rapid declines and “sudden” death. Ramorum blight causes shoot-tip dieback and leaf spots in woody shrubs and ornamental trees. Unlike sudden oak death, ramorum blight rarely kills its host (Grünwald et al., 2008).

Discovery and Spread

Observers first spotted symptoms of sudden oak death and ramorum blight in the mid-1990s in Marin County, California, and in European nurseries (Mascheretti et al., 2009). In 2000, researchers identified the common causative fungal pathogen for these diseases, *P. ramorum* (Garbelotto and Rizzo, 2005; Kliejunas, 2010). Trade and transportation of ornamental plants enabled the introduction of this novel pathogen into the United States and Europe. *P. ramorum* has since spread north to southern Oregon and south to Big Sur in California, where tree mortality rates are among the highest (Kliejunas, 2010). In 2009, symptoms of the disease were first detected in Japanese larch trees in the English counties of Devon, Cornwall, and Somerset (Forestry Commission, 2010). By August 2010, the disease had spread to Japanese larch trees in the counties of Waterford and Tipperary in Ireland (Brasier and Webber, 2010; Forestry Commission, 2010). Many now consider this disease to be a serious threat to Japanese larch and possibly other tree species in Europe (Brasier and Weber, 2010).

The ornamental trade and other anthropogenic factors continue to serve as a source for global and local spread of *P. ramorum*. In 2004, “pre-symptomatic” *Rhododendron* plants infected with the fungal pathogen were discovered in a

⁶⁴ See contributed manuscript by Rizzo in Appendix A (pages 312–324).

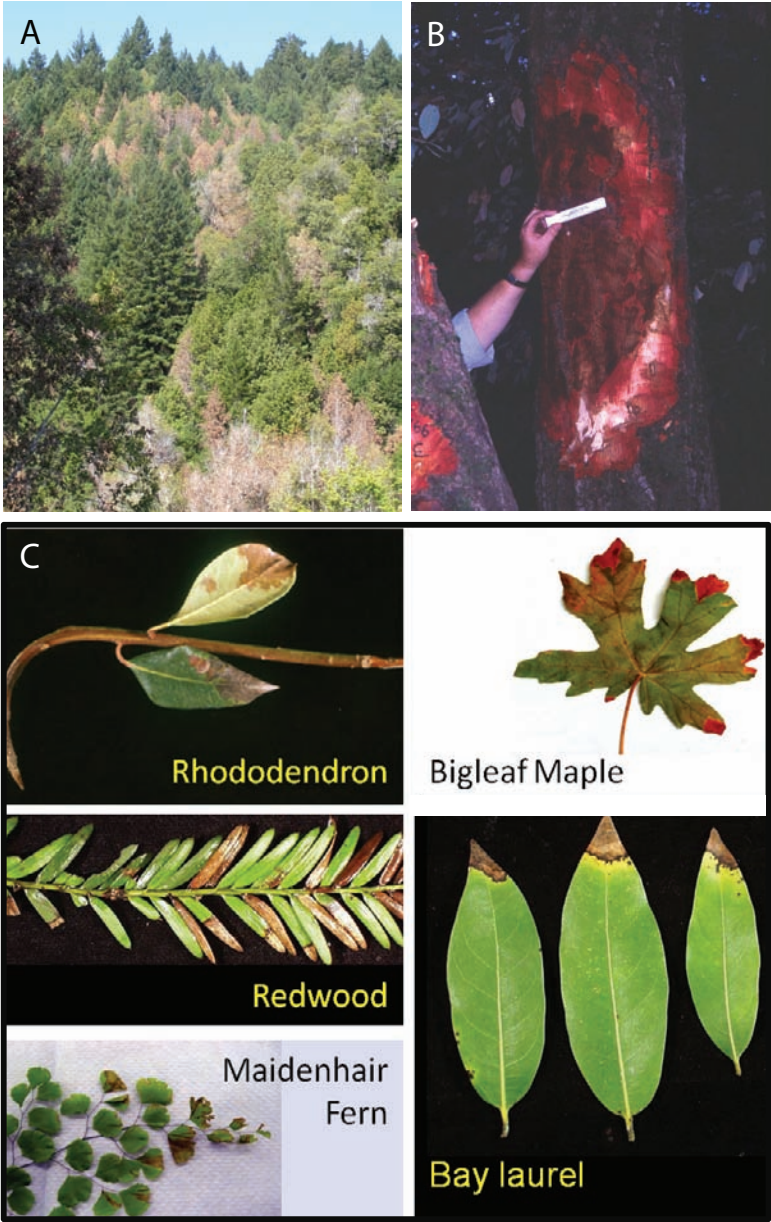


FIGURE WO-19 Sudden oak death and ramorum blight. (A) Aerial view of a forest in Humboldt County with patches of trees dying of sudden oak death; (B) canker on tanoak; (C) signs of ramorum blight on a variety of host plant species.
SOURCE: Rizzo (2010).

TABLE WO-2 Disease Types and Associated Symptoms Caused by *P. ramorum*

Disease	Symptoms	Host Categories	Typical Hosts*	Geography and Environment
Sudden oak death	Stem cankers; bleeding cankers	Forest trees; garden trees	Coast live oak, tanoak, European beech	North American forests; European gardens
Ramorum blight	Foliar and twig blight; tip and shoot dieback; leaf blight	Ornamental trees and woody shrubs; forest understory plants	Viburnum, rhododendron, pieris, lilac; coast redwood, Douglas fir; tanoak, California bay laurel	European nurseries and gardens; North American nurseries and forest

* Only a small selection of typical hosts is presented. For a complete list of hosts refer to Grünwald et al., 2008, and references within.
SOURCE: Adapted from Grünwald et al. (2008).

Southern California plant nursery, but not before the nursery had shipped potentially infected plants to more than 40 states (Figure WO-20) (Goss et al., 2009).

As Rizzo noted, many of the fungicides used in the nursery trade do not kill the pathogen, but rather suppress symptoms; this is probably how *P. ramorum* spreads over very long distances via the plant trade. Leaves, flowers, and stems of infected plants carry the pathogen, which is also spread by the transportation of plant or associated plant materials, including soil (Kliejunas, 2010). Waterways are an effective means of spreading *P. ramorum*. Investigators have detected the pathogen in streams contaminated with run-off from infected nurseries (Kliejunas, 2010). *P. ramorum* may also provide an interesting case study of humans acting as vectors for fungal disease, as sudden oak death “has potentially been spread to new areas by hikers, mountain bikers, and equestrians” (IOM, 2010). Rizzo also pointed to the movement of “green waste,” that is, compost, firewood, mulch, and other plant matter, as another possible means of spreading the fungus within and across ecosystems.

As of 2009, the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service reported detection of *P. ramorum* in 11 states (Alabama, California, Georgia, Maryland, Mississippi, New Jersey, North Carolina, Oregon, Pennsylvania, South Carolina, and Washington) at 30 sites (24 nurseries and 6 in the landscape) (Kliejunas, 2010). The pathogen’s eastward spread has placed Eastern native forests at risk and has led many experts to worry that *P. ramorum* could have ecological consequences comparable to those of chestnut blight and Dutch elm disease (Goss et al., 2009).

The distribution of sudden oak death in California forests is heterogeneous, Rizzo noted. Weather—including winds, temperature, humidity—contribute to the pathogen’s establishment and spread within a new environment (Kliejunas,

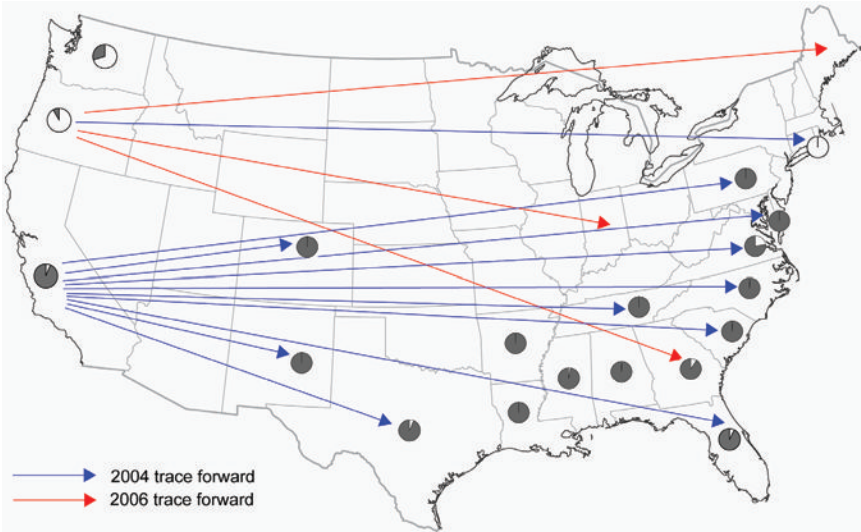


FIGURE WO-20 *P. ramorum* “migration” pathways. The ornamental plant trade continues to serve as a major pathway for the spread of *P. ramorum*. Arrows indicate confirmed *P. ramorum*-positive nursery trace forwards. Blue arrows are 2004 trace forwards and red arrows are 2006 trace forwards. There were no confirmed trace forwards in 2005 or 2007. Pie charts show the distribution of the two groups of isolates (of NA 1 lineage) among sampled states.

SOURCE: Goss et al. (2009).

2010). Infected tanoaks do not contribute significantly to disease spread, but, as Rizzo observed, many other plants in forested areas (e.g., understory and canopy trees) are also host species for *P. ramorum*. The pathogen’s very broad host range, therefore, is also an important transmission factor for disease. In California forests, the California bay laurel (*Umbellularia californica*) drives transmission of the pathogen (Kliejunas, 2010; Rizzo and Garbelotto, 2003). Leaf tips of these trees serve as a prolific source of inocula. Dissemination of spores can occur via wind and rainsplash (Rizzo and Garbelotto, 2003). In the United Kingdom, Japanese larch trees not only developed canker and died but the leaves also served as foliar hosts and the source of massive amounts of inocula (Brasier and Weber, 2010).

Origins

The lack of reports of *P. ramorum* in the United States before the mid-1990s, combined with its aggressiveness and limited geographic range relative to its hosts’ distribution, suggests that *P. ramorum* was only recently introduced

into the United States (Grünwald et al., 2008). Genetic evidence supports the hypothesis that the U.S. and European strains are distinct and that both strains likely originated from a third, as yet unknown, source (Grünwald et al., 2008). Molecular studies have identified three main lineages: (1) EU1—found in both North American and European nurseries and some European woodlands; (2) NA2—found in California and Washington nurseries; and (3) NA1—found in North American nurseries and in California and Oregon forests (Grünwald et al., 2008).

An alternative explanation is that *P. ramorum* may have existed in California for many years, but only recently emerged because of changes in the environment (e.g., increased temperatures, fire suppression, modifications in land use patterns) that have led to an increased prevalence and aggressiveness of the pathogen (Rizzo and Garbelotto, 2003). Native *P. ramorum* or other *Phytophthora* sp. may also have evolved into a more virulent form, may have undergone a change in host specificity or preference, or may represent an entirely new hybrid species (Rizzo and Garbelotto, 2003). It has been reported that a novel *Phytophthora* hybrid—a cross between *P. cambivora*, an oak pathogen, and *P. fragariae*-like isolates, a strawberry pathogen—emerged in Europe in the 1990s and has killed thousands of alder trees (*Alnus* spp.) (Brasier et al., 1999). Given the large number of *Phytophthora* spp. in California agricultural and horticultural environments, a hybrid origin for *P. ramorum* is certainly a possibility; although other explanations are also possible (Rizzo and Garbelotto, 2003; Tyler et al., 2006).

The Rapid Global Spread of Yellow “Stripe” Rust on Wheat

The presence of two virulent and highly aggressive yellow rust strains at high frequencies at epidemic sites on five continents may represent the most rapid and expansive spread ever of an important crop pathogen. This epidemic trend may continue because the aggressive strains, which can tolerate higher temperatures, are still evolving.

—Hovmøller et al. (2010, p. 369)

Yellow “stripe” rust on wheat has recently reemerged as a major threat to global food security (Hovmøller et al., 2010). This destructive, cooler climate, wheat disease can spread rapidly via wind and human activities locally and globally. Between 2000 and 2002, two new and highly aggressive⁶⁵ strains of the associated fungus (*Puccinia striiformis* f. sp. *tritici*) appeared on three continents—North America, Australia, and Europe—causing record wheat crop losses (Hovmøller et al., 2010; Milus et al., 2009). As of 2009 these new strains of yellow rust had spread to major wheat-growing areas in the Middle East, North

⁶⁵ Speaker Mogens Hovmøller defined “aggressiveness” as the quantitative ability to cause more disease, more quickly, on a susceptible host.

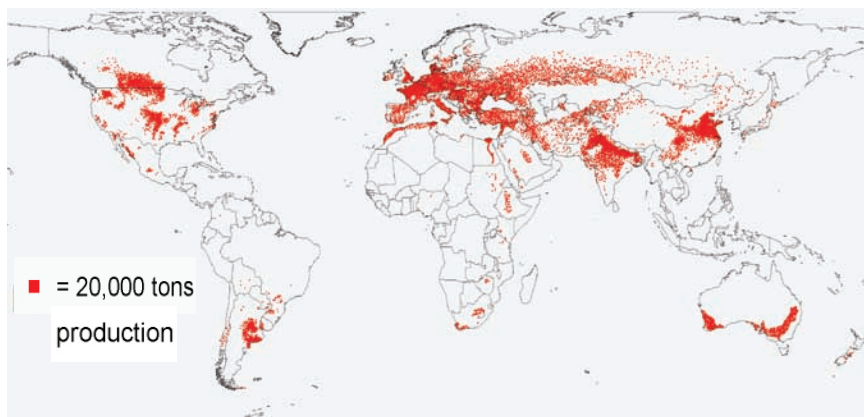


FIGURE WO-21 Wheat production regions worldwide. Each red dot represents 20,000 tons of wheat production.

SOURCE: Trethowan et al. (2005).

and Eastern Africa, Western and Central Asia, and China (Hovmøller et al., 2010). According to Hovmøller et al. (2010), this may be the most rapid and expansive spread of an important crop pathogen ever documented.

Wheat is the most widely grown cereal crop, produced as food for humans and as feed for livestock (Figure WO-21). Worldwide, wheat accounts for one fifth of the total human caloric intake. In regions such as Western Asia, it can account for as much as half of the daily calorie intake (Hovmøller, this volume; Stone, 2010). Epidemics of rust disease have been held in check by rust-resistant wheat cultivars developed in the mid-20th century, and other agricultural practices (Koerner, 2010). Since 2000, however, the natural history⁶⁶ of yellow rust appears to have changed. Disease is now emerging in regions previously considered inhospitable to this fungal pathogen (Hovmøller et al., 2008; Milus et al., 2009).

Phenomenology

Puccinia striiformis f. sp. *tritici* (hereafter, *P. striiformis*) is an obligate, basidiomycetous, pathogen of wheat. It infects the green tissues of host plants, causing damage to leaf blades, and reducing the yield and quality of produced grains and seeds (Chen, 2005). Severe infections of yellow rust also stunt the growth of wheat plants. Average reported yield losses due to yellow rust range from 10 to 70 percent; yield losses in highly susceptible cultivars can reach 100 percent (Chen, 2005). Weather—including humidity, rainfall, temperature, and

⁶⁶ The natural development of something (as an organism or disease) over a period of time.

wind—is critical for developing favorable conditions for fungal infection and growth (Chen, 2005).

Named for the yellow pustules of powdery spores (urediniospores) that appear as “stripes” on the leaf blades of infected plants, yellow “stripe” rust is an important “cooler climate” wheat disease (Chen, 2005) (Figure WO-22). Disease can occur early in the growing season, when temperatures are low. Areas affected by this disease tend to be in temperate regions and high-elevation areas in the tropics (Chen, 2005). *P. striiformis* depends on a living host for survival and produces huge numbers of airborne spores that are carried by wind from one susceptible host to another (Brown and Hovmøller, 2002).

Discovery and Spread

The most widespread yellow rust epidemic in the United States occurred in 2000 (Milus et al., 2006). Before that time, yellow rust in the United States was restricted to the temperate regions of California and the Pacific Northwest, where cool and moist weather patterns prevail (Chen, 2005). Suddenly, “overnight, more or less,” remarked Mogens Støvring Hovmøller of Aarhus University, the disease appeared in the warmer and drier wheat belt. (Dr. Hovmøller’s contribution to the workshop summary report can be found in Appendix A, pages 252–263.) For the first time, severe epidemics were reported east of the Rocky Mountains—in South Dakota, Nebraska, Kansas, Oklahoma, and Texas (Figure WO-23) (Milus et al., 2009).

Similarly, between 2002 and 2003, yellow rust swept across Western Australia, where yellow rust was absent until 2002—an area once considered too warm for severe epidemics of yellow rust (Milus et al., 2009). In central and northern Europe, epidemics on wheat at that time were less pronounced, the majority remaining resistant to the disease. According to Hovmøller, two *P. striiformis* strains of almost identical DNA-fingerprints were responsible for these epidemics (Hovmøller et al., 2008). Human activity was likely the main driver for pathogen introduction in North America and Australia, Hovmøller remarked, given the rapid spread of the pathogen to distant continents in less than 3 years and the indistinguishable DNA fingerprints of prevalent pathogen isolates. Once the pathogen arrived, the disease spread rapidly via airborne spores, he said. According to Hovmøller, the presence of large areas of susceptible crop varieties likely amplified and accelerated the spread of these yellow rust strains.

The emergence of yellow rust in new warmer regions concerns researchers because it suggests a change in its epidemiology. Indeed, the strains of *P. striiformis* associated with recent outbreaks of disease are more aggressive,⁶⁷ more heat tolerant, and able to produce two to three times more spores in less time than other strains (Milus et al., 2009). These adaptations appear to improve the fitness

⁶⁷ Able to cause more disease more quickly on susceptible host plants.



FIGURE WO-22 Yellow “stripe” rust on wheat.
SOURCE: Hovmøller (2010).

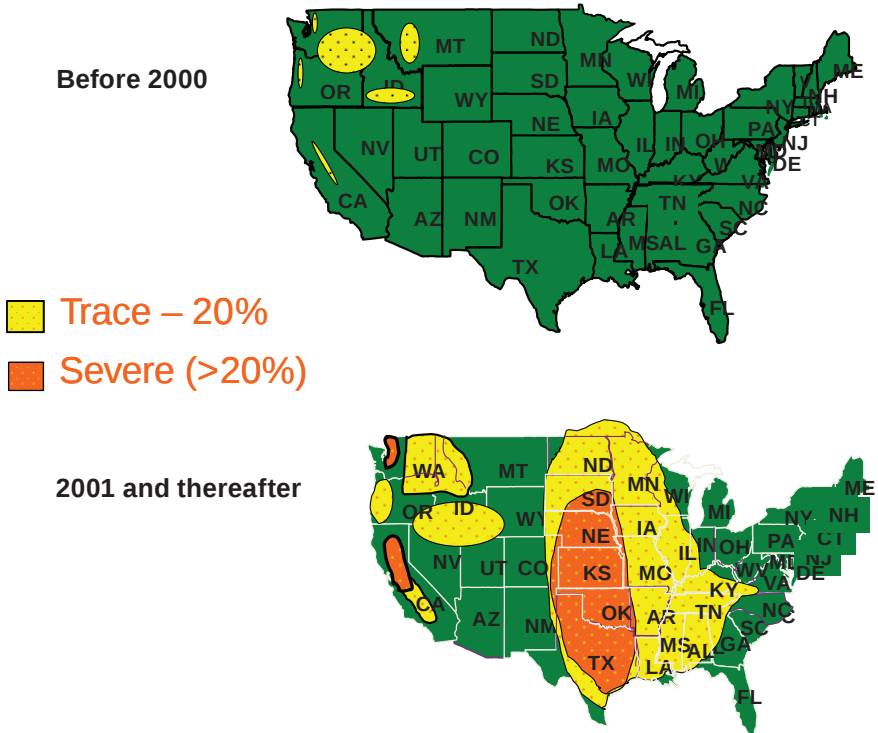


FIGURE WO-23 Presence of “trace” and “severe” levels of yellow rust in North America since 2000.

SOURCE: Adapted from Chen (2005).

of these strains and may explain their rapid spread on a global scale (Hovmøller et al., 2008). In Australia and the United States, these newer, more aggressive strains appear to have replaced older strains and have continued to thrive in subsequent seasons (Milus et al., 2009). According to Hovmøller, while the epidemic has apparently slowed down in North America from 2006 to 2009, major yellow rust epidemics occurred in northern and eastern Africa, Western and Central Asia, China, and the Middle East (Hovmøller et al., 2010). In 2010, according to Hovmøller, observers reported disease outbreaks in northern and eastern Africa, Asia, and the Middle East, and the epidemics reappeared in the United States.

Origins

The exact geographic origin of these more aggressive strains of *P. striiformis* is unknown, although phylogenetic analyses reveal that West and Central Asia may be the evolutionary origin. Hovmøller remarked that the dramatic change in

phenotype (e.g., spore production, heat tolerance), coupled with the sudden appearance of these strains suggests a recombination event somewhere, rather than evolution through a series of mutations.

Unlike many of the fungal diseases discussed at the workshop, yellow rust is well known. Epidemics of this disease have plagued the world's farmers for centuries, and *P. striiformis* is familiar to plant breeders. In the 1940s, Norman Borlaug⁶⁸ developed new “rust-resistant” wheat strains that also dramatically increased global crop yields (Rust in the bread basket, 2010). These advances inspired the “Green Revolution” that brought these techniques and disease-resistant wheat into widespread use. According to Brown and Hovmøller (2002), these techniques have helped to keep many crop diseases under control, but now, relatively few crop varieties (with specific resistance genes) are sometimes used across large areas at “continental scales.” A reduced crop diversity in modern agriculture, according to Hovmøller, increases the potential impact posed by the global movement of new, more virulent forms of plant pathogens (Brown and Hovmøller, 2002; Stukenbrock and McDonald, 2008).⁶⁹

SURVEILLANCE, DETECTION, AND RESPONSE

Fungal diseases are an emerging threat to human, animal, and plant health—not simply because of the morbidity and mortality associated with these infections, but also because of the limited means and capabilities to rapidly detect and diagnose these diseases and the lack of effective tools for disease mitigation and treatment. Detection of many emerging fungal diseases—such as amphibian chytridiomycosis, WNS, and *C. gattii*—has relied on the astute observer in the field. However, once these diseases have become established in a new environment, the spread of fungal pathogens is limited by the environmental constraints imposed on them, such as temperature, humidity, drought, or moisture. Therapeutic interventions and management strategies for these diseases remain limited—underscoring the urgent need for active disease surveillance and additional research to better understand and address fungal disease threats. As observed by Forum member Kevin Russell of the Department of Defense's Global Emerging Infections Surveillance and Response System, the fungal world

⁶⁸ Norman Borlaug was an American agronomist. His work to develop disease-resistant crop strains earned him the titles of Nobel laureate and “the father of the Green Revolution.” Borlaug was one of only six people to have won the Nobel Peace Prize, the Presidential Medal of Freedom, and the Congressional Gold Medal. He was also a recipient of the Padma Vibhushan, India's second highest civilian honor.

⁶⁹ In 1999 a new, highly virulent, strain of wheat stem “black” rust, Ug99, emerged in Uganda. This pathogen was able to circumvent the genetic resistance of wheat hybrids developed during the Green Revolution. This pathogen has now spread to Kenya, Ethiopia, Sudan, Yemen, and Iran (Vurro et al., 2010). Researchers worry that Ug99 will soon spread to the major wheat growing regions of Pakistan and India, which account for ~20 percent of the world's wheat supply (Vurro et al., 2010).

is too huge, too unknown, and too threatening not to develop improved capacity for detection, diagnosis, and response to emerging fungal pathogens and diseases.

David Blehert, joined by Forum member Russell and fellow Forum member Jacqueline Fletcher of Oklahoma State University, emphasized the importance of cross-disciplinary communication and a “One Health”⁷⁰ approach for developing a more robust capacity for global disease surveillance, detection, and response. As noted by Bartlett, the detection and response to the emergence of *C. gattii* in British Columbia and the U.S. Pacific Northwest in 1999 ultimately involved professionals and expertise from the veterinary, medical, public health, and plant and wildlife communities. Indeed, because of its close interactions with plants and ability to cause disease in humans and animals, *C. gattii* was called the “poster child” for a One Health approach.

The plant health community was considered by many at the meeting to be an equal partner in a One Health approach to infectious disease detection and response. Fungi can form associations with and, in some cases, be pathogenic to humans, animals, *and* plants, Fletcher observed. She described the need to gain a better understanding of how fungal pathogens might “jump” between plant and animal or human systems. Others remarked on the indirect, but potentially significant, impacts of plant pathogens on human health and well-being, including threats to ecosystem stability or global food security. As Fletcher noted, however, “plant pathogens have only been incorporated into current One Health initiatives [in] a very minor way.”

Surveillance Networks

Over the past several decades, various systems for passive and active surveillance for emerging and reemerging diseases of humans, animals, and plants have been developed at regional, national, and international levels (GAO, 2010). Systems supporting the surveillance and detection of emerging fungal plant pathogens are the most sophisticated, possibly due to the historical importance of fungal diseases on economically important foodstuffs, crops, and plants (IOM, 2007; Rossman, 2009).

Disease surveillance and detection in the United States is a shared responsibility of various state and federal programs (Figure WO-24) (GAO, 2010). The Centers for Disease Control and Prevention (CDC) and other federal agencies, including the USDA, Department of Defense (DoD), and Department of the Interior (DOI), independently gather and analyze national infectious disease surveillance reports as well as morbidity and mortality data for humans, plants, livestock, and

⁷⁰ One World, One Health® is a registered trademark of the Wildlife Conservation Society and reflects the need to establish a more holistic approach to preventing epizootic disease and for maintaining ecosystem integrity for the benefit of humans, their domesticated animals, and the foundation biodiversity that supports us all. For more information, see <http://www.oneworldonehealth.org/> (accessed April 11, 2011).

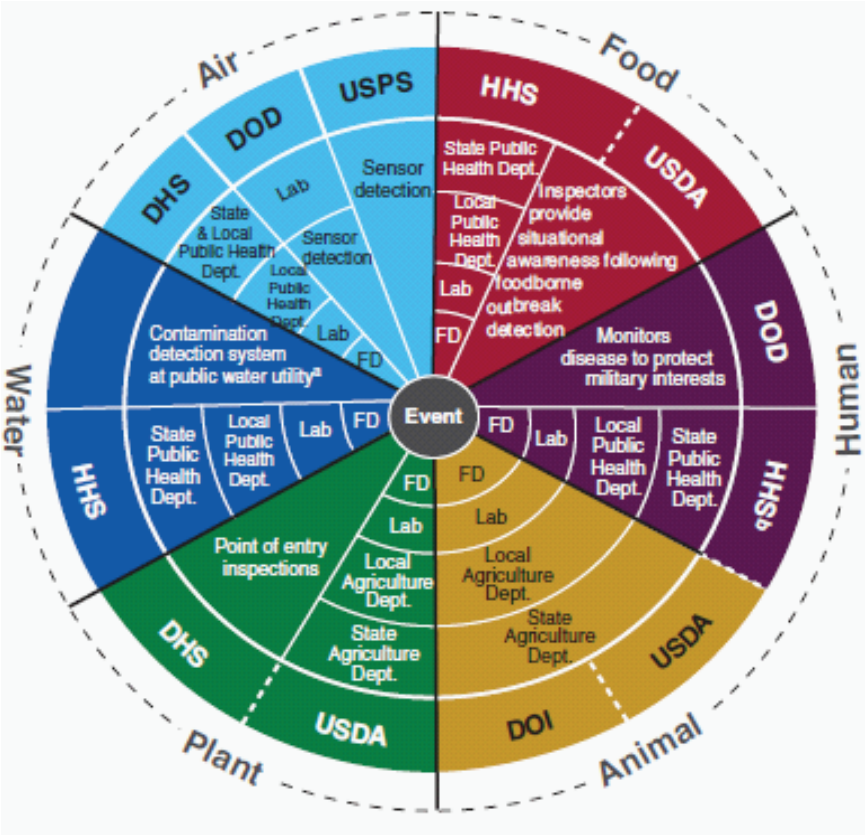


FIGURE WO-24 Roles and responsibilities for monitoring for pathogens in humans, animals, plants, food, and the environment in the United States.
SOURCE: GAO (2010).

wildlife. The CDC, USDA, DoD, and DOI independently fund and maintain both domestic and international laboratory networks for infectious disease diagnostics (Choffnes, 2008; GAO, 2010). Reporting and verification of outbreaks of specific diseases of concern or of unusual symptoms or health disturbances takes place at the state level. The findings are sent to federal agencies for further investigation, and if appropriate, the coordination of a response to the potential disease threat (GAO, 2010).

The DOI includes the U.S. Geological Survey (USGS) National Wildlife Health Center (NWHC), which is tasked with providing information, technical assistance, research, education, and leadership on national and international wildlife health issues. According to Blehert, WNS surveillance is currently largely opportunistic, based upon people making unusual observations in the field, then

sending bats to the NWHC or other laboratory for diagnostic investigation. Federal, state, and local government officials have taken several steps to try to curb the spread of WNS and prevent additional bat deaths. In 2009, the U.S Fish and Wildlife Service issued a cave advisory that established guidelines for entering bat hibernacula, that issued recommendations for decontamination of caving gear, and that asked researchers and spelunkers not to bring clothing or equipment that has been used in caves from affected areas to caves in unaffected areas (FWS, 2011). Federal and state agencies have also closed caves on public lands in order to prevent people from inadvertently spreading the fungus to new areas (FWS, 2011). Total funding for WNS research from federal and state agencies increased from approximately \$1.8 million to \$10 million between fiscal year 2007 and fiscal year 2010.

Formal global surveillance programs—tracking the emergence and reemergence of microbial threats to human, animal, and plant health—are coordinated by the World Health Organization (WHO), the World Organisation for Animal Health (OIE), and the Food and Agriculture Organization (IOM, 2007). WHO manages the Global Outbreak Alert and Response Network which partners with 120 “informal” (as discussed below) and “formal” (e.g., regional WHO offices, government, military, or university research centers) information sources to identify and respond to disease outbreaks (Heymann and Rodier, 2004). OIE manages an international reporting system on animal disease that includes reporting of “exceptional epidemiological events” and periodic gathering of animal health information (Jebara, 2004). Forum member Peter Daszak of the EcoHealth Alliance noted that the responsibility for infectious disease surveillance of wildlife could be undertaken by the United Nation’s International Union for the Conservation of Nature (IUCN). The IUCN has developed a working group of 400 wildlife specialists from around the world.⁷¹

International Regulations and Coordination

International regulations that support infectious disease surveillance and detection activities include: the International Health Regulations (IHRs) and the Sanitary and Phytosanitary Measures (Baker and Fidler, 2006; Cash and Narasimhan, 2000; MacLeod et al., 2010; WHO, 2008). In 2008, the OIE added amphibian chytridiomycosis to its list of “notifiable”⁷² aquatic animal diseases, (Schloegel et al., 2010). As several participants observed, however, this report-

⁷¹ For more information, see http://www.iucn.org/about/work/programmes/species/about_ssc/specialist_groups/specialist_group_profiles/veterinary_sg_profile/.

⁷² Within the OIE, a notifiable disease is one whose “detection must, by mandate, be notified by the competent veterinary authority to the OIE as required under Chapter 1.1 of the Aquatic Code. OIE members are also required to report the presence or absence of each disease in their territory on a semi-annual basis, and ensure disease surveillance programs are implemented to support any claims of freedom from one or both diseases” (Schloegel et al., 2010, p. 4).

ing requirement only applies to OIE member states, and only to animals traded internationally. Indeed, the effectiveness of these formal and informal reporting regimes has yet to be demonstrated, and many have suggested that fear of adverse economic consequences (e.g., trade and tourism restrictions) will limit their usefulness as an early warning disease reporting network (Cash and Narasimhan, 2000; Fidler, in IOM, 2010; Hueston, in IOM, 2007; Perrings et al., 2010).

Guidelines on hygiene and quarantine procedures for captive and wild animals have also been developed by the conservation community to reduce the spread of zoonotic diseases, but these guidelines are considered underused or difficult to enforce (Daszak et al., 2000). Two conventions developed to address the international wildlife trade and the conservation of biodiversity, the Convention for International Trade of Endangered Species and the Convention on Biological Diversity are based entirely on voluntary agreement, noted speaker Weldon. While these agreements reflect noble aspirational goals, according to Weldon, there is limited opportunity to actually implement the measures.

Several participants emphasized the limitations of current capacity to detect emerging pathogenic fungi. As speaker Fisher observed, national strategies are limited by their focus on known threats to humans and agriculturally important species, and international strategies are nearly nonexistent or very slow moving. This is particularly true of wildlife surveillance, which Fisher said is “completely under the radar.” Forum member Roger Breeze of Lawrence Livermore National Laboratory agreed, noting that “we are not very good at looking for things we know about, even those diseases that are economically important, such as foot and mouth disease.” Breeze continued that “what we are talking about over the last few days is broadening the number of organisms involved and the number of areas of economic life that are involved.” He went on to note that many organisms discussed during the workshop, such as ornamental plants, do not currently fall under any one organization’s regulatory responsibility. “We have a huge international failure in biosecurity,” according to Breeze, and the problem “needs to be approached in a different manner.”

No single agency or multilateral organization is solely focused on infectious diseases in humans, plants, and animals. Several workshop participants observed that the creation of a single entity that was responsible for collecting and analyzing data from across the “threat” spectrum and ensuring that disease interventions are based on the input of professionals working with humans, domestic animals, and wildlife could significantly enhance current disease surveillance and response capabilities (Choffnes, 2008; GAO, 2010; Hueston, in IOM, 2007; Perrings et al., 2010).

Improved coordination of disease surveillance and response activities, Daszak noted, would “benefit all sectors—whether it is food production, travel and trade, or human and environmental health.” Moreover, sectors may benefit in unanticipated ways. Blehert remarked that “wildlife health is important to world health. Not just with regard to disease surveillance, but also with regard to basic

research. There is much that we can learn from emerging diseases of wildlife such as WNS or amphibian chytridiomycosis that likely have significant implications with regard to ecosystem integrity and function. Only by incorporating domestic animal health, wildlife health, and human health into the same model can we fully understand the ecology of infectious disease.”

Several participants suggested that within the United States, an interagency task force could link together the plant, animal, and human health communities. Forum member Russell added that the Department of Defense is now one of several interagency partners involved in a forum on emerging pandemic threats as a sub-Interagency Policy Committee (IPC) of the U.S. government’s Global Health Initiative (GHI). Among its other activities, this sub-IPC assembled an interagency working group that developed a document detailing the U.S. response to the revised IHRs. He suggested that this interagency forum could serve as an effective model for coordinating the U.S. government activities in areas of common concern.

Forum member Edward McSweegan, from the National Institutes of Allergy and Infectious Diseases, added that a previous interagency program that was focused on international infectious diseases was orchestrated by the U.S. Department of State and the Office of Science and Technology Policy (OSTP). Forum Vice-Chair, James Hughes of Emory University, noted that this effort was established under the aegis of the Committee on International Science, Engineering, and Technology Policy of President Clinton’s National Science and Technology Council and involved many agencies: the National Institutes of Health (NIH), CDC, Food and Drug Administration, U.S. Agency for International Development, and DoD, among others. He said that many of the recommendations from their 1995 report are “still relevant to today’s world.”⁷³ McSweegan also suggested that the funding of cross-disciplinary research on emerging fungal diseases might be modeled after the success of the NIH–National Science Foundation (NSF) Ecology of Infectious Diseases Initiative,⁷⁴ perhaps as a collaboration of the NIH, USGS, and USDA.

Informal Disease Reporting Networks

Informal disease reporting networks are an increasingly important component of global disease surveillance. Examples include ProMED-mail, which is administered by the International Society for Infectious Diseases, and Bd-Maps, which was developed at Imperial College London. Both are platforms that allow

⁷³ For more information see http://clinton1.nara.gov/White_House/EOP/OSTP/CISSET/html/exsum.html#top.

⁷⁴ For more information on the joint NIH–NSF Ecology of Infectious Diseases Initiative, visit the website http://www.fic.nih.gov/programs/research_grants/ecology/index.htm.

contributors anywhere in the world to report and access disease observations—even via cell phone applications (Brownstein et al., 2009; Fisher et al., 2009).

Since its founding in 1994, ProMED-mail⁷⁵ (Program for Monitoring Emerging Diseases, PMM) has served as an important platform for rapid communication about emerging infectious diseases of humans, animals, and plants (IOM, 2007). Speaker Larry Madoff, of the Massachusetts Department of Public Health and University of Massachusetts Medical School, explained that in contrast to the traditional, hierarchical approach to public health reporting, ProMED collates information from a wide variety of unofficial or informal sources⁷⁶ and distributes reports to members in near real time (Brownstein et al., 2009; Madoff, 2004; Morse et al., 1996). A recent quantitative assessment of the effect of informal source reporting on the global capacity for infectious disease detection concluded that ProMED-mail and other informal disease reporting resources improve the timeliness of detection and reporting, although the effect varies geographically (Chan et al., 2010).

ProMED-mail is a free service, with all reports screened by a panel of expert moderators before being posted to over 54,000 subscribers from more than 180 countries. ProMED-mail emphasizes a One Health approach to disease surveillance, Madoff remarked, by reporting on plant (mostly threats to food crops), animal (both agricultural and zoonotic threats), and human pathogens to all subscribers. Pointing to *C. gattii* as an example of a recently emerged fungal human pathogen with many non-human hosts, Madoff reminded the audience that the risk of disease emergence in humans is greater among pathogens with many non-human hosts (Woolhouse and Gowtage-Sequeria, 2005).

Informal efforts to aggregate information on the presence or absence of disease in the field also contribute to improving the speed and broadening the scope of current disease surveillance. Fisher described the *Bd* Global Mapping Project⁷⁷ (*Bd*-Maps) and its associated activity, RACE (Risk Assessment of Chytridiomycosis to European amphibian biodiversity). *Bd*-Maps collects information from groups of researchers in the field and national surveillance data from several European countries, including the Netherlands, Spain, Switzerland, and the United Kingdom, to create a shared database that provides information on where *Bd* has been detected, globally and locally. Fisher hopes this information will not only aid in the prediction of where *Bd* will likely emerge in the future, but also encourage at-risk areas to implement appropriate biosecurity controls.

The *Bd*-Maps project has a public website with a map detailing the incidence of positive *Bd* reports and, for each report, links to data. There is also an embargoed (private) website to encourage participation of scientists who prefer that their data remain private. As of December 2010, the publicly available database

⁷⁵ See <http://www.promedmail.org>.

⁷⁶ Including clinician reports, blogs, chat rooms, websites, news media, YouTube videos, and other Internet sources.

⁷⁷ See at www.bd-maps.net.

reported about 6,500 *Bd*-positive animals out of 30,000 sampled among 3,500 sites worldwide, with 49 of 74 countries and 440 species of amphibians with known *Bd* infections. The data come from multiple sources, including contributions directly from the field using a smart phone application called EpiCollect.⁷⁸ Fisher observed that these data may be used to assess either global or country-level trends and to detect broad-level associations (e.g., the data show that *Bd* is present in many areas where species richness has declined without any [other] explanation). Fisher noted that the project has provided a means for communicating important information rapidly among interested parties.

Predictive Modeling

Surveillance and response efforts could be better targeted to at-risk populations or circumstances through the use of mathematical models and Geographical Information Systems (Weinberg, 2005). Several workshop participants described the use of predictive modeling as a way to “get ahead” of the spread of invasive fungal diseases into new and highly susceptible regions:

- Rödder et al. (2009) developed a model based on taxa susceptibility to *Bd*, biogeographic, basic biology, environmental, and demographic data to illustrate which regions of the world are more at risk for *Bd* among amphibians than others.
- Meentemeyer et al. (2004) and Václavík et al. (2010) identified a number of areas in California and Oregon, respectively, currently unaffected by sudden oak death, but that are at high risk based on host species distributions, climate suitability for pathogen transmission (e.g., rain), and other factors.
- Kelly et al. (2005) used the agreement of multiple models to develop a risk map for the development of sudden oak death in the United States based on information on nationwide vegetation/host (hardwood diversity and hardwood density), topography, and climate (e.g., precipitation, frost days, temperature, and many other layers) (Figure WO-25).
- Mak et al. (2010) demonstrated that data derived from environmental sampling (in native vegetation, soil, air, and water), combined with animal and human surveillance data, could be used to predict *C. gattii* occurrence. The methodology employed, ecological niche modeling, yielded very accurate predictions for *C. gattii* in British Columbia, with animal surveillance data in particular being a good indicator of *C. gattii* in an area (Mak et al., 2010).

⁷⁸ EpiCollect allows global positioning systems-localized data to be submitted by phone to a common web database (see Aanensen et al., 2009).

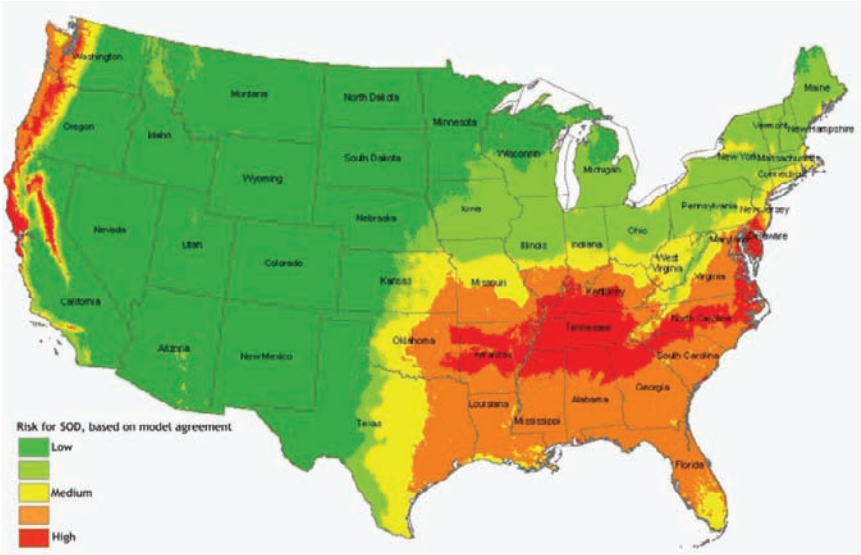


FIGURE WO-25 Risk for sudden oak death in the continental United States based on agreement among five spatially referenced models.
 SOURCE: U.S. Department of Agriculture, Forest Service (Kelly et al., 2005).

Daszak discussed the use of predictive models to get ahead of disease emergence entirely by anticipating where viral pathogens of zoonotic origin are most likely to emerge in the future. The PREDICT project is part of the U.S. Agency for International Development Emerging Pandemic Threat program.⁷⁹ PREDICT uses wildlife surveillance data and models to identify (1) geographic hot spots for the emergence of infectious disease, and (2) species that may serve as reservoirs of disease.⁸⁰ Daszak noted that the prediction models developed for the PREDICT project were grounded in wildlife surveillance data that included the active collection of tens of thousands of samples from wildlife among 24 countries. Any newly discovered viruses in these samples are deemed “high priority” if they appear to be closely related to other known viral pathogens. High-priority pathogens are further characterized and, if appropriate, people who interact with the wildlife that may be affected by these pathogens are educated and advised to avoid contact. While PREDICT is currently focused only on viral pathogens, Daszak observed that the same approach could be used for fungi and fungal pathogens.

⁷⁹ For more information, see: http://www.usaid.gov/our_work/global_health/home/News/ai_docs/emerging_threats.pdf.

⁸⁰ See contributed manuscript by Daszak in Appendix A (pages 188–196).

Detection and Diagnosis

As many participants noted, improving the capacity for detecting fungal disease threats relies on having trained and acute observers in the field as well as a better understanding of the “baseline” of fungal diversity and distribution. Methods for the “discovery” of fungi and fungal pathogens are also needed. As speaker Rizzo noted, “We can put things on lists, but those are the things we know about. The big problems are the ones we don’t know about.” Similar challenges were identified for disease diagnosis.

Astute Observers in the Field

Human capacity is needed for more effective surveillance and detection of fungal and other infectious diseases of humans, animals, and plants, remarked many participants. Weldon noted the importance of field biologists in discovering the accelerated loss of amphibian biodiversity and in initiating investigations on the possible causes—from climate change and habitat destruction to chemical pollutants. Blackwell remarked that the causative agent of amphibian chytridiomycosis (*Bd*) was recognized as a fungus rather late in the epidemic by one of the few mycologists who study the fungal phylum Chytridiomycota. Howlett urged more training in classical mycology: “While molecular systematics and phylogenomics has helped to advance understanding of mycology, these methods need to be complemented by field studies and identification of the causative agent of a disease by symptoms or pathogen morphology.” Forum member Fletcher agreed, adding that more classically trained plant pathologists are needed: “These are the scientists who can go out into the field and identify pathogens of any type, fungal or otherwise.” Many senior plant pathologists are near retirement, but not many younger pathologists have the skills and knowledge to take their place, she said. Rizzo agreed, noting that in the 1980s, seven researchers in California specialized in forest diseases; by the time that sudden oak death emerged as a major problem in California, there were none. He further observed that agricultural departments in colleges across the United States continue to be downsized.

Speakers Galgiani and Holland emphasized the importance of improved education of physicians and other front-line healthcare workers in the diagnosis and treatment of fungal diseases. Holland noted that fungal diseases due to previously undiagnosed primary immune deficiencies are not frequent, but that a few cases happen “every year, in every country.” He further cautioned that, “if you don’t think about them and you don’t recognize these diseases as fungal, the patients don’t survive.” Even in areas with endemic fungal disease, the correct differential diagnosis is often missed, commented Galgiani. In the case of symptomatic Valley Fever, which often initially presents as a community-acquired pneumonia, specific antibody testing is required to discriminate *Coccidioides* infections from other causes of pneumonias. Even then, Galgiani noted, with early infections conventional serological testing produces false positives in an estimated one third to two thirds of all infected patients.

Rapid and Accurate Tools for Detection and Diagnosis

The lack of sensitive and specific tools for the diagnosis of emerging diseases limits the effectiveness of disease surveillance and treatment efforts (IOM, 2007). In the ornamental plant trade, surveillance focuses on preventing the introduction of plant pathogens. This occurs at the international, national, and regional levels, Rizzo explained. In Europe and North America, strict controls have been placed on nurseries to quickly contain and eradicate outbreaks of *P. ramorum* (Kliejunas, 2010). In the United States, plants in nurseries are subject to routine inspections, and plants that are sold across state or county boundaries are closely monitored. In states such as Oregon, Washington, and California, they are quarantined to ensure they are free from infection (Kliejunas, 2010). Despite these measures, *P. ramorum* continues to spread via ornamental plant trade pathways (see sudden oak death case example). In part, this is due to the lack of effective detection tools. Asymptomatic infections and the use of fungicides can limit the effectiveness of quarantine protocols based only on visual inspection.

To track the spread of the *C. gattii* outbreak, public health officials in British Columbia and the U.S. Pacific Northwest have listed *C. gattii*-associated disease as a reportable disease. However, speaker Julie Harris of the CDC remarked that *C. gattii* surveillance has been limited by overreporting of the most severe cases and underreporting of all cases. In cases where samples are sent to the laboratory for identification, not all labs are using the canavanine-glycine-bromothymol blue (CGB) agar test⁸¹ or genetic sequencing⁸² that are needed to differentiate between *C. gattii* and *C. neoformans* infections. Harris noted that underreporting is due to a number of factors including the fact that many of the smaller laboratories across Washington and Oregon:

- may not be aware of the need for culture to make an accurate diagnosis;
- may not be aware that they should be sending isolates to their respective state health departments for confirmatory testing; or
- may lack the capacity for fungal culture and testing.

Harris observed that labs with mycology capacity are not as common as labs with viral or bacterial capacity, and requests for additional training or capacity need to come from the local level. She noted that the *C. gattii* Public Health Working Group, formed in 2008 by the CDC and state and local public health departments and laboratories and the British Columbian Centre for Disease Control, is working to standardize surveillance by increasing clinician awareness of *C.*

⁸¹ During a canavanine-glycine-bromothymol blue agar test, *C. gattii* grows and the medium changes color; *C. neoformans* does not grow, and the medium remains a light green color.

⁸² Bartlett pointed out that because of the close evolutionary relationship between the two species, much of the early literature on the outbreak refers to the outbreak pathogen by its former name, *C. neoformans* var. *gattii*.

gattii infection and working with global laboratories to characterize genetic and phenotypic variety in *C. gattii*.

Padilla, from the Smithsonian Conservation Biology Institute (SCBI), identified fungal diagnostic capacity as a particularly challenging area of emerging fungal disease threat management in wild animals and one in need of further research. Too often, new fungal pathogens in wildlife are either misdiagnosed or undiagnosed. The limited diagnostic capacity leads to the “clumping” of information under known fungal disease syndromes, Padilla remarked, and this often precludes the recognition of true emerging fungal diseases and prevents further investigation of true host–pathogen dynamics. Fungal infections are often identified only to the genus, not the species level, making it difficult to understand host–species relationships. The clumping of information could also result in dangerous management decisions—when assumptions about one host are based on what is known about another host. For example, he reported that preliminary findings by researchers working at the SCBI suggested that the bacterium *Janthinobacterium lividum* does not provide the same anti-chytridiomycosis protection in *Bd*-infected captive-bred Panamanian golden frogs (*Atelopus zeteki*), as it does in *Bd*-infected yellow mountain frogs (*Rana muscosa*). In fact, *J. lividum* seems limited in its ability to colonize the skin of *A. zeteki* and thus is also limited in its ability to play the same anti-fungal role that it does in *R. muscosa*. However, the understanding of a bacterium–host system conferring anti-fungal protective properties suggests that other species-specific host-adapted bacteria could confer the same protection to *A. zeteki*. Padilla expressed hope that these findings will lead to more appropriate treatments for this particular frog species.

The Fungal “Background”

Limited understanding of fungal biodiversity and biogeography can impede surveillance, detection, and discovery efforts, noted Blehert. It has been reported that an abundance of closely related *Geomyces* species have been found in the same soil that harbors *G. destructans* (Lindner et al., 2010). These related species are currently confounding the ability to conduct routine soil analysis as a mode of surveillance for WNS, Blehert said. Rizzo added that “when we find something new, it’s very difficult to know whether it is exotic and something to be concerned about or an interesting new native organism that we weren’t aware of.”

Russell asked participants for their views on effective techniques for the “discovery” of fungal pathogens (i.e., detection of an unknown disease agent). Heitman suggested that the fungal nuclear ribosomal internal transcribed spacer (ITS⁸³) sequences might be useful for determining if genetic material isolated by researchers is fungal in origin. Blackwell mentioned that mycologists now use

⁸³ ITS sequences are sections of non-functional RNA that are highly variable even between closely related species and are widely used for taxonomic purposes (e.g., Cendejas-Bueno et al., 2010; Garner et al., 2010; Leaw et al., 2006).

non-culture-based molecular tools coupled with field explorations to identify new fungal species (Jones et al., 2011; Jumpponen and Jones, 2009; Lara et al., 2010; Porter et al., 2008; Schadt et al., 2003) and to characterize the distribution of fungal species (Daughtrey et al., 1996). These techniques are increasingly being used because many fungal species are not easily cultured. Culturing techniques and pathology investigations, however, are still needed to characterize an organism: these are “hard and laborious things to do,” observed Daszak, but the “value of the product is so much better, it is orders of magnitude better, because you can do something with it.” You can “send it to others for consultation or determine an organism’s biology or how it causes disease in host species,” Daszak said.

Blackwell noted that the NSF has had several programs in systematics⁸⁴ and biodiversity for some years. In 2004, the NSF created the “Assembling the Tree of Life” program with the goal of constructing an evolutionary history for all major lineages of life (See glossary for more information). These and other related programs have increased support for research on the evolution and diversity of Kingdom Fungi, which has been helpful for improving detection, diagnosis, and discovery methods (see Blackwell et al., 2006; Hibbett et al., 2007). A database of 40,000 fungal species (with an emphasis on fungal plant pathogens) developed by the USDA Agricultural Research Service includes information such as host range, geographic distribution, relevant scientific literature, and for some species, descriptions and illustrations (Rossman and Palm-Hernandez, 2008).

Treatment and Response

Active surveillance and early detection of emerging fungal diseases are important and partially effective. However, due to the magnitude of trade in plants and plant products, “[pathogens] are getting through, and they are going to get through; we are not going to stop that” as Stack remarked. Attention is also needed to developing ways to respond to fungal disease threats (e.g., effective and economical treatment options) and to recover from emerging fungal diseases.

Responding to Fungal Diseases of Plants—from Agriculture to Landscapes

In plants, disease eradication strategies include clear-cutting or controlled burning of infected plants (Rizzo et al., 2005). Fungicides are often used to protect high-value plants, but their widespread and frequent use is often not economically feasible (Rizzo et al., 2005; Scheffer et al., 2008). The development of resistant cultivars or strains, which may take years to decades, is currently the most successful disease control strategy in plants, particularly agricultural crops (Vurro et al., 2010).

⁸⁴ The study of the general principles of scientific classification, and the classification of organisms according to the presumed, natural, and evolutionary relationships among them.

Developing strains of wheat that are resistant to the newly emerging and more aggressive forms of yellow rust is the primary strategy for limiting the devastating effects of *P. striiformis* on wheat. In the meantime, early detection is essential to reduce crop yield losses due to yellow rust, Hovmøller said. In the short term, options for control are limited to fungicide sprays which may be unavailable or not affordable to farmers in the developing world. The replacement of susceptible wheat with locally adapted, resistant, or less susceptible varieties can also slow disease spread, he remarked.

As Hovmøller noted, when it comes to wheat rust, “what’s going on in one continent may be your problem the following day.” To prevent long-term damage, intensified international collaboration is needed to build wheat rust surveillance, detection, and response capacity. Several promising developments on the international scale were reported by Hovmøller. In 2008, a Global Rust Reference Center (GRRC) for yellow rust was established to improve yellow rust management in countries where facilities and expertise are scarce. GRRC is supported by Aarhus University in Denmark, the International Center for Agricultural Research in the Dry Area, and the International Maize and Wheat Improvement Center (CIMMYT). In 2011 the activities will be extended to wheat stem rust (*Puccinia graminis*) via projects facilitated by the Borlaug Global Rust Initiative. GRRC is complementing existing national diagnostic laboratories, which cannot receive rust samples year round from all countries. The primary goals of GRRC are to conduct virulence and race⁸⁵ analyses, secure isolates for future resistance breeding and research, facilitate research and training, and provide information for a global wheat rust early warning system. The Borlaug Global Rust Initiative, which was established in response to the stem rust Ug99 outbreak in East Africa, now deals with all three wheat rusts.⁸⁶

Disease management is considerably more difficult when dealing with plants of limited economic value (i.e., non-timber and non-crop plants), despite their significant ecological value, Rizzo noted. Management of sudden oak death and ramorum blight, according to Rizzo, is “scale dependent.” One can manage individual trees, landscapes, or entire regions (Rizzo et al., 2005). At the individual level, fungicides are available that can be injected into a tree or sprayed on the bark to prevent infection (Garbelotto et al., 2002). In forests, containment, including cutting and controlled burnings in areas with infected trees, is the primary means of infection control. When asked whether there is a possibility for developing treatments for sudden oak death that could be applied at the landscape level, Rizzo said the options are limited. In Oregon, there have been attempts to conduct aerial spraying with phosphonate (a chemical fungicide), but it is unlikely that any type of aerial spraying would be acceptable in California. Rizzo also cautioned that it took many decades to breed genetic resistance for Dutch

⁸⁵ A subspecies group of pathogens that infect a given set of plant varieties (Cornell University, plant pathology glossary).

⁸⁶ For more information see www.globalrust.org.

elm disease. The complexity of oak genetics also makes it very challenging to use breeding to develop oaks resistant to *P. ramorum* infection. Rizzo went on to note that research on potential biocontrol agents, such as viruses, is at a very early stage.

Landscape-level management also uses predictive modeling. For sudden oak death, models based on host species distribution, climate, and other factors identify areas at risk for invasion by the pathogen. These areas can then be surveyed using “aerial imaging, plot-based monitoring, and stream sampling to determine the presence of *P. ramorum* or signs of infected trees” (IOM, 2008b). Eradication methods are only effective if the disease is detected early enough. For areas where the pathogen is established, management approaches seek to avoid negative ecological consequences, such as the growth of invasive plant species. Ultimately, Rizzo said, we are trying to develop methods to “live with the pathogen.”

Treatment Options for Fungal Diseases of Humans and Animals

Available antifungal therapies are generally of limited value due to toxicity problems (Figure WO-26) (Ostrosky-Zeichner et al., 2010). The lack of accurate diagnostics further limits the effectiveness of existing fungal treatments. Approaches using antibody therapy and vaccines (for certain endemic pathogens) remain challenging due to the ongoing evolution of pathogens (Cox and Magee, 2004; Galgiani, 2007, 2008; Ostrosky-Zeichner et al., 2010). Overall, there are few new therapeutic agents in the development pipeline with the potential for broad antifungal effects (Ostrosky-Zeichner et al., 2010).

The development of new treatments for fungal diseases has also been slowed by an underappreciation for the effects that fungal diseases can have on human health, Galgiani asserted. In endemic areas of Arizona and California, about a third of all coccidioidomycosis cases lead to illnesses requiring medical attention⁸⁷ (Tsang et al., 2010). While oral therapy with azole antifungal drugs is safe and convenient, many patients do not respond to treatment (20–40 percent failure rate). Moreover, many patients who initially respond to treatment experience relapses after treatment ends (Galgiani, 2007; Hector and Laniado-Laborin, 2005). Galgiani reviewed current efforts at the University of Arizona to develop a new antifungal known as nikkomycin Z, a competitive inhibitor of chitin synthase that interferes with cell wall construction (Galgiani, 2007). Discovered in an antifungal discovery program by Bayer in the 1970s, the compound demonstrated antifungal activity in mice in the 1980s (see Hector et al., 1990). Only after the

⁸⁷ Valley fever is often dismissed as a self-resolving mild illness. In fact valley fever can be long-lasting and have a tremendous impact on activity levels (see Galgiani, 2007). Recent surveillance activities conducted by the Arizona Department of Health Services, in collaboration with the CDC, reported that coccidioidal illness lasted an average of 6 months, with 75 percent of workers taking more than one month of sick leave and 40 percent of infected persons requiring at least one night of hospitalization at some point during the course of their illness (Tsang et al., 2010). Annual hospital costs alone amount to nearly \$90 million (\$86 million in 2007; Tsang et al., 2010).

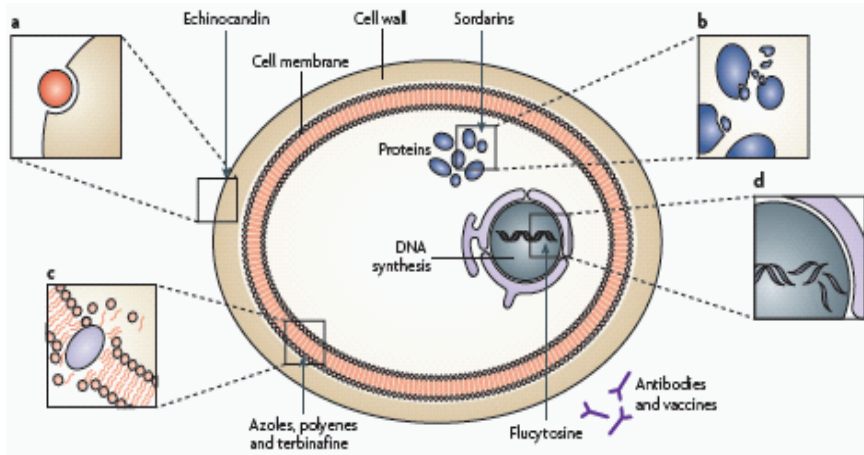


FIGURE WO-26 Mechanisms of action of selected antifungals. An illustration of the mechanism of action of currently available antifungals as well as selected antifungals under development. (A) Echinocandins and nikkomycin Z inhibit the formation of the fungal cell wall. (B) Sordarins interfere with protein assembly. (C) Azoles, polyenes, and terbinafine disrupt the fungal cell membrane. (D) Flucytosine interferes with DNA synthesis. Antibodies and vaccines prevent fungal infection or block and/or destroy the fungal cells. SOURCE: Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Drug Discovery (Ostrosky-Zeichner, L., A. Casadevall, J. N. Galgiani, F. C. Odds, and J. H. Rex. 2010. An insight into the antifungal pipeline: Selected new molecules and beyond. *Nature Reviews Drug Discovery* 9(9):719–727).

University of Arizona acquired the compound in 2005, have clinical trials resumed (Galgiani, 2007).⁸⁸

In the absence of a vaccine or other preventive measures for *C. gattii* infection of humans and animals, officials concede the public can do little to protect themselves from infection (Knox, 2010). Additional research is needed to clarify the epidemiology and drug susceptibilities of the various strains of *C. gattii* present in the region to help inform treatment guidelines. Moreover, researchers need to learn more about the natural history and pathogenicity of the fungus to further prevention, treatment, and intervention efforts (Datta et al., 2009a,b). Heitman noted that research is ongoing to determine the nature of hypervirulence (D'Souza et al., 2011); the differences between immune responses to *C. gattii* and *C. neoformans* infections (Cheng et al., 2009); and why *C. gattii* can so readily invade the cells of immunocompetent individuals (Kronstad et al., 2011; Ma et al., 2009; Voelz and May, 2010).

⁸⁸ See contributed manuscript by Galgiani in Appendix A (pages 196–207).



FIGURE WO-27 Frogs in the Sierra Nevada region, being treated in baths containing a fungicidal bacterium in hopes of eliminating infection by the fungal pathogen (*Bd*) associated with the deadly disease: amphibian chytridiomycosis.

SOURCE: Photo by Anand Varma.

The challenge of developing effective treatments for fungal diseases is compounded by the scale and complexity of treating diseases of wildlife. Fungicidal treatment protocols are being explored for amphibian chytridiomycosis and include methods to alter the skin microbiome⁸⁹ (Fisher et al., 2009; Harris et al., 2009) (Figure WO-27). For WNS, some researchers are investigating whether treating bats with antifungal agents might improve their survival (Platt, 2010), while others are exploring the possibility of developing a vaccine against this fungal pathogen (Buchen, 2010). Adapting these protocols to large and dispersed wild animal populations, while minimizing unanticipated ecosystem impacts, is challenging and may continue to limit conservation efforts (Fisher et al., 2009). In addition to captive breeding programs, conservation efforts target habitat preservation, limiting the spread of infected species, and protecting endangered species. Further research is also needed to fill in some gaps in knowledge that still exist. Researchers need to gain a better understanding of the biology of both *Bd* and *G. destructans* and their respective hosts to answer questions related to the determinants of virulence, the hallmarks of effective immune response, and the specific

⁸⁹ Fungicides, and more recently cutaneous bacteria of amphibians (e.g., *J. lividum*) known to produce an antifungal metabolite, are applied to the skin of amphibians.

mechanisms by which these pathogens kill their hosts. Added to this is the unique challenge of managing disease in hibernating animals in delicate underground ecosystems. Biology of infectious diseases, however, is not part of the traditional wildlife ecology education curriculum, Blehert remarked. Nor are speleologists, tourists, recreational cavers, or hikers required to have such knowledge.

Buying Time Through the Conservation of Threatened Wildlife Populations

Captive breeding programs at the SCBI⁹⁰ have helped to rescue species that were on the verge of extinction, endangered by habitat loss or by the introduction of disease into areas with naïve and susceptible host populations. According to Padilla, captive propagation programs can also serve two additional functions in the management and mitigation of emerging fungal diseases⁹¹:

- Fungal diseases identified in a captive animal population can be an early indication of an emerging threat in the wild. Based on Padilla's experience, there is a wide range of opportunistic and primary fungal diseases that have been observed in captive animals.
- Captive populations are also established for the purpose of studying a fungal disease that would otherwise be difficult or impossible to study. An example of this important work is the captive population of Japanese giant salamanders (*Andrias japonicus*) established by the Smithsonian National Zoological Park, in which the presence of *Bd* and the efficacy of itraconazole treatment can be studied and monitored over time in ways that could not be possible in their wild counterparts.

SCBI has used captive breeding to save several species from extinction. The endangered black-footed ferret population was revived from just 18 individuals in 1988 to a current population of 800 to 1,000 in the wild (Weidensaul, 2000). Other animal species, including the golden lion tamarin, California condors, Przewalski's horses, and the scimitar-horned oryx, have also benefited from the SCBI's captive breeding program's success.

Despite success with multiple species, establishing and maintaining captive breeding programs is technically challenging. In the fall of 2010, SCBI developed a captive colony of the endangered Virginia big-eared bats (*Corynorhinus townsendii virginianus*) in response to the threat posed by *G. destructans*. Specialist insect-eating bats, such as the Virginia big-eared bat, are notoriously difficult to keep in captivity. But, Padilla noted, in light of the possible extinction of this endangered subspecies, SCBI decided to take on the "high risk" project

⁹⁰ Formerly known as the National Zoo's Conservation and Research Center, the SCBI is an umbrella organization for the Smithsonian's global efforts to conserve species and train future generations of conservationists. See <http://nationalzoo.si.edu/scbi/default.cfm>.

⁹¹ See contributed manuscript by Padilla in Appendix A (pages 296–312).

of developing a captive colony of these bats. However, Padilla said, although the bats did not die of WNS, the colony of 40 bats experienced extremely high mortality (90 percent) in the first 200 days of captivity.

The Amphibian Ark⁹² is a global network of captive breeding programs working in the short term to protect amphibian species at immediate risk of extinction (IUCN, 2005). The Smithsonian's National Zoo currently houses a fifth of the world's Panamanian golden frog populations (Figure WO-28). It is hoped that the Smithsonian's expertise in captive breeding will contribute to the preservation of amphibians and New World bats that are currently at risk of local or global extinction.

Weldon highlighted a number of successful conservation programs that target amphibian populations outside of captive breeding programs.⁹³ These included:

- The population management of *Alytes obstetricans* in Peñalara Natural Park, Spain, in response to annual *Bd* outbreaks;
- Australia's *Bd* Threat Abatement Plan, which was initiated in 2006, with the goal of preventing amphibian populations and regions that are currently free of chytridiomycosis from becoming infected; and
- Madagascar's Early Detection Plan, which monitors high-risk areas (e.g., ports of importation, areas visited by tourists, areas of high biodiversity) and builds facilities for captive breeding and research in the event that *Bd* does reach the island (Weldon et al., 2008).

Importantly, Weldon noted, these programs include disease prevention as a prioritized conservation measure.

Weldon also recounted one instance in which conservation efforts unintentionally contributed to the spread of diseases. Population decline of amphibians on the island of Mallorca were linked to *Bd* infection in 2008 (Walker et al., 2008). The "source" of infection was traced to a project designed to boost populations of the island's midwife toad (*Alytes muletensis*). Cross-contamination is thought to have occurred between two species that were cohoused at the breeding facility: the midwife toad and imported frogs from South Africa (*Xenopus gilli*) that were infected with *Bd*. Captive midwife toads reintroduced into the wild served as vectors that brought the pathogen to other amphibian populations on the island (Rosenblum et al., 2009; Walker et al., 2008). Weldon said, "This illustrates that if we are to proceed with the reintroduction programs, great caution should be taken, because you could potentially be introducing the pathogens with the species that you are trying to conserve."

⁹² The Amphibian Ark carries out the ex situ components of the Amphibian Conservation Action Plan developed by the World Conservation Union. For more information, see <http://www.amphibianark.org/pdf/ACAP.pdf> and www.amphibianark.org.

⁹³ See contributed manuscript by Weldon and Fisher in Appendix A (pages 355–367).



FIGURE WO-28 Panamanian golden frog (*Atelopus zeteki*). The Smithsonian National Zoo has established a captive breeding program to help rescue this critically endangered species.

SOURCE: Photo by Brian Gratwicke, Wikimedia commons.

Prospects for Preventing and Managing Emerging Fungal Diseases

Although participants described a number of challenges in efforts to detect and respond to emerging fungal pathogens, discussion also revealed many opportunities to better prevent and manage these threats. Daszak stressed the need to “focus on the underlying causes, because they cross all the kingdoms. The drivers of plant disease also drive the emergence of disease in wildlife and humans: travel, trade, agriculture, deforestation, and other environmental disturbances.” While improving capacity to prevent and manage disease emergence is an enormous undertaking and a long-term endeavor, Daszak stressed that “the evidence and data on steps that can be taken are there; it is just a matter of turning our knowledge into action.”

To many participants, a better understanding of the ecology of fungi and fungal disease seemed paramount (Rizzo, 2005). To better manage outbreaks of infectious disease, scientists may also benefit from a greater understanding of biological invasions in all their variety and complexity. The incipient “cross-fertilization” of ecology and epidemiology offers support for such investigations, as does the growing recognition of the interdependence of human, animal, and plant health, and of the central importance of the environment in influencing host–pathogen interactions (Scholthof, 2007). The following strategies and areas

of focus for preventing and managing all types of biological invasions, including fungal pathogens, were discussed during the workshop as particularly promising:

- Anticipating invasions based on global and local trade patterns (Dybas, 2004);
- Identifying and interrupting routes of transport that represent high risk for biological invasions of all kinds, rather than focusing on individual species or known diseases (Rossman, 2009);
- Recognizing the importance of human travelers as disease couriers, transmitters, and sentinels and, therefore, a critical target for infectious disease surveillance and detection (Pimentel et al., 2005);
- Recognizing that domestic animals, wildlife, and plants can also serve as important “asymptomatic” carriers or sentinels for disease and are also an important target for disease surveillance and detection efforts;
- Prescreening imported plants and animals that are likely to become problematic invasive species (O’Donnell, 2006);
- Establishing the prevention of the spread of invasive species as an international public good, which requires coordination among nation states (Keller and Perrings, 2010). Because such a system is only as strong as the “weakest link,” efforts are also needed to assist developing nations in establishing capacity for surveillance, detection, and prevention of biological invasions (Keller and Perrings, 2010);
- Educating the public and inspectors at airports and seaports about the environmental and economic threats posed by invasive species, and the role of tourism and travel in biological introductions (Pimentel et al., 2005);
- Focusing efforts on markets (e.g., wildlife markets) to regulate, reduce, or eliminate trade that threatens the health of humans, domestic animals, wildlife, and ecosystems (Karesh et al., 2005);
- Developing bioeconomic models to assess the economic impact of the introduction of invasive species and of alternatives for their prevention and mitigation (Evans, 2003);
- Increasing capacity for the early detection of, and rapid response to, biological invasions (Dybas, 2004); and
- Applying mathematical models to forecast the worldwide spread of infectious diseases, identify endangered regions, and analyze potential control strategies (Hufnagel et al., 2004; Weinberg, 2005).

Each of these approaches supports the overall goal of identifying and exploiting common characteristics of invasive animals, plants, and microbes in order to reduce their impact. To pursue this strategy requires “a new perspective, a new thinking, a consideration of all alien introductions in a deliberate, truly comprehensive system,” ecologist Richard Mack has observed (Dybas, 2004, p. 618). “If we do that, then we will have a sound science-based policy.”

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Appendix A

Contributed Manuscripts

A1

THE EMERGENCE OF *CRYPTOCOCCUS GATTII* IN BRITISH COLUMBIA AND THE PACIFIC NORTHWEST¹

Karen H. Bartlett, Sarah E. Kidd, and James W. Kronstad²

An unprecedented emergence of cryptococcal infections in animals and otherwise healthy humans was recognized in 1999 on the east coast of Vancouver Island, British Columbia. Unexpectedly, these infections were caused by *Cryptococcus gattii*, a species closely related to the AIDS-associated fungal pathogen *Cryptococcus neoformans*. Human cases have continued over the past 8 years and now total approximately 170 with eight deaths. Extensive environmental

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++ Of major importance

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sampling, coupled with detailed molecular typing of isolates, revealed areas of permanent and transient colonization with primarily three genotypes of the fungus. *C. gattii* was found in air, soil, water, and in association with numerous tree species. Importantly, there is solid evidence for human-mediated dispersal of the pathogen, and *C. gattii* has now been detected in the environment on the mainland of British Columbia and in the Pacific Northwest. Associated animal and human cases are now being reported and further spread of the pathogen may be inevitable.

Introduction

The basidiomycetous yeast *Cryptococcus neoformans* has a global distribution and has achieved prominence in recent decades because of its propensity to infect immunocompromised people (Casadevall and Perfect, 1998). In fact, cryptococcosis is recognized as an AIDS-defining illness, and in the absence of highly active antiretroviral therapy, the disease is a significant cause of death in individuals with HIV infection (Bicanic and Harrison, 2005; Bicanic et al., 2005). People and animals acquire the fungus via the inhalation of desiccated yeast cells or basidiospores from environmental sources such as avian guano, soil, and trees. Pulmonary infection often results in dissemination to the central nervous system and *C. neoformans* is the leading cause of fungal meningitis (Casadevall and Perfect, 1998).

Isolates of *C. neoformans* have previously been divided into three varieties known as *grubii*, *neoformans*, and *gattii* and into serotypes (A–D and hybrids such as AD) defined by antigenic differences in the capsular polysaccharide that is the major virulence factor (Casadevall and Perfect, 1998). The *gattii* variety is now recognized as a separate species based on phenotypic and molecular traits, and mating (Kwon-Chung et al., 2002). Thus the current view is that the species *C. neoformans* (var *grubii* and *neoformans*) contains strains of serotypes A, D, and AD, and the distinct species *C. gattii* contains isolates of the B and C serotypes (Kwon-Chung and Varma, 2006). An excellent review of the differences between *C. gattii* and *C. neoformans* has been published by Sorrell (Sorrell, 2001).

Extensive surveys have been performed over the past 10 years to characterize the genotypes and distribution of *C. neoformans* and *C. gattii* isolates (Barreto de Oliveira et al., 2004; Boekhout et al., 2001; Boukhout et al., 1997; Fraser et al., 2005+; Kidd, 2003; Kidd et al., 2004 ++; Kidd et al., 2005+; Meyer et al., 1999; Meyer et al., 2003). These surveys used a variety of DNA-based typing methods to provide detailed classifications of isolates into molecular types. Thus, isolates of *C. neoformans* var *grubii* (serotype A) are represented by the VNI, VNII, and VNB (Litvintseva et al., 2006) molecular types, var *neoformans* (serotype D) is represented by the VNIV type, and isolates of the AD hybrid serotype are the VNIII type. Four molecular types are recognized for *C. gattii* isolates (designated VGI–VGIV) and further divisions within the molecular types have been identified

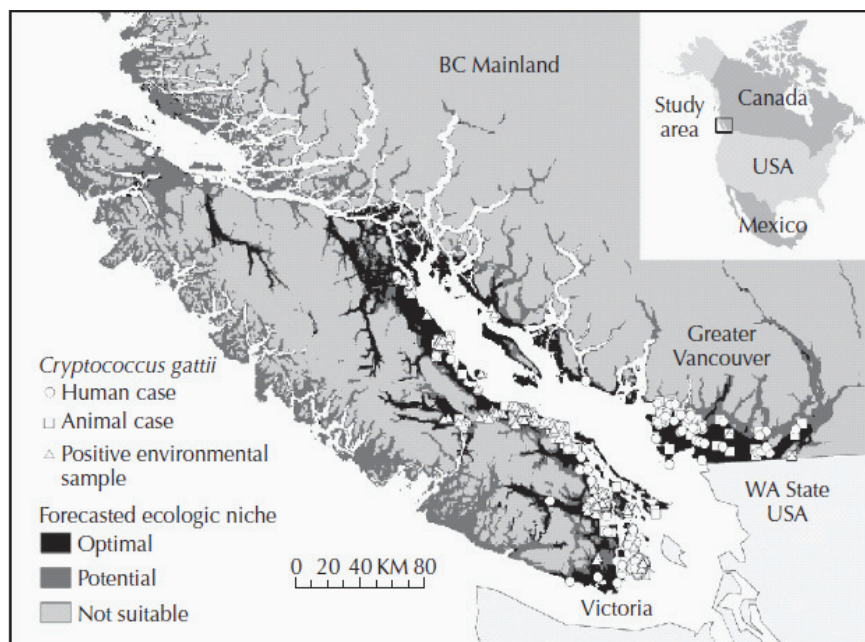


FIGURE A1-1 Map of the forecasted ecologic niche and region of emergence of *C. gattii* in British Columbia (BC). The optimal, potential, and unsuitable ecologic niches of *C. gattii* in BC are indicated based on biogeoclimatic data for the region (Mak, 2007). Note that the distribution of human and animal cases and the locations of positive environmental samples coincide primarily with the optimal ecologic niche. The information on human and animal cases, and environmental sampling, from Washington (WA) is not included.

(Fraser et al., 2005+; Kidd et al., 2005+; Kidd et al., 2007++). For example, VGII strains can be further classified into VGIIa and VGIIb subtypes, as well as other less-well characterized subtypes (Kidd et al., 2004; MacDougall et al., 2007++).

There is currently an intense focus on *C. gattii* due to the unprecedented emergence of the VGI, VGIIa, and VGIIb molecular types as primary pathogens of humans and animals on Vancouver Island in British Columbia (BC) (Kidd et al., 2004; MacDougall et al., 2007++) (Fig. A1-1). Remarkably, the majority of human cases have occurred in people without recognized immunologic defects, thus highlighting the unusual pathogenicity of *C. gattii* relative to *C. neoformans*. The purpose of this review is to summarize recent progress in the investigation of this fascinating emergence with regard to human and animal exposure, environmental colonization, isolate characterization, and the potential for further dispersal.

Overview of Veterinary and Clinical Aspects of the Emergence of *C. gattii* in BC

Animal sentinels played a key role in the study of the emergence of *C. gattii* in BC and in particular contributed to our understanding of the range of environmental niches for the pathogen. A single veterinary pathology laboratory handled clinical specimens from the majority of southern BC veterinary practices, and this allowed early detection and monitoring of *C. gattii* in the animal population. In addition, the BC Provincial Animal Health Branch Laboratory was able to perform necropsies on porpoises that were found stranded and dead on Vancouver Island and nearby islands, and these became index cases (Stephen et al., 2002). Beginning prior to the first documented human case in 1999 and continuing to the present, veterinary cases have been diagnosed two to three times more frequently than human cases (Lester et al., 2004); this disparity is likely an underestimate given that only those animals seen by a veterinarian are diagnosed and that infections in wildlife are not considered. The diagnosed cases have primarily been in companion animals (dogs, cats, and ferrets) but also include other domesticated species such as llamas, horses, mink, and psittacine birds (Duncan et al., 2006b; Lester et al., 2004; Stephen et al., 2002). Sampling in the environs of these animal cases has been particularly productive for identifying sources of *C. gattii* (Kidd et al., 2007a++; MacDougall et al., 2007++).

Unlike the colonized koalas of Australia (Krockenberger et al., 2002), no significant wild animal host or reservoir has been identified in BC. Limited surveys of wild animals were performed between 2003 and 2007 with the examination of necropsy samples of nares, lung, anus or cloacae, and brain for *C. gattii*. In two surveys, all fatally injured animals turned into rescue facilities were studied. In the first study, 91 animals (14 species) were examined, and only two eastern gray squirrels were positive (Duncan et al., 2006a). In the second study, only one great blue heron was found to have a pulmonary *C. gattii* infection of 226 animals necropsied (Bartlett, unpublished data). Additionally, 18 river otters were trapped in early spring 2007, but none showed signs of disease or colonization with *C. gattii* (Bartlett and Balke, unpublished data). Duncan et al. (2005b) established sentinel veterinary practices in areas known to have exposure to airborne *C. gattii* and found positive *C. gattii* cultures from nasal swabs of asymptomatic animals in 4.3% of 94 cats, 1.1% of 280 dogs, and 1.5% of 351 horses. Additionally, six cats and two dogs were found to have cryptococcal antigen titers of greater than 1:2. Of seven cats and five dogs that were selected from the asymptomatic but culture- or antigen-positive cohorts and followed over 27 months, only two cats progressed to clinical disease, suggesting that the majority of animals exposed to *C. gattii* may naturally clear the organism (Duncan et al., 2005a).

In the first years of recognition of both the emergence of *C. gattii* disease and the stability of the pathogen's environmental niche, it appeared that all human and animal cases had some contact with Vancouver Island. MacDougall and Fyfe (MacDougall and Fyfe, 2006) were able to identify human cases of disease

with historic travel to Vancouver Island and to determine a likely incubation period (median 6–7 months) based on isolated exposure. In addition, Hoang et al. (Hoang et al., 2004) performed a retrospective chart review examining all cases of cryptococcosis identified between 1997 and 2002 at the largest teaching hospital located on the BC mainland. They discovered that there had been a sudden increase in cryptococcal cases of all origins (*C. neoformans* var *grubii*, *C. n.* var *neoformans*, *C. gattii*, and *C. laurentii*), but all *C. gattii* cases (3/26 charts) reported travel history to Vancouver Island (Hoang et al., 2004). The first cases of mainland-acquired *C. gattii* infection were identified in animals (ferret, llama, and cats) in 2003, and three cases in cats in Washington were reported in 2005. Eight off-island human cases with no travel history to an endemic area were documented (five in BC and two in Oregon) in 2004 to 2005 (MacDougall et al., 2007++). Upton et al. (Upton et al., 2007+) recently reported the first confirmed human case in Washington presenting in 2006, and the Whatcom County Public Health Department has now identified four additional cases diagnosed in 2007 (Stern, personal communication). Unlike in BC, cryptococcosis is not yet a reportable disease in Washington, although public health officials are actively soliciting case studies. The VGIIa genotype accounted for 78% of the examined veterinary cases and 87% of the human cases; all off-island veterinary cases to date had the VGIIa genotype (Bartlett, unpublished data) (MacDougall et al., 2007++).

Environmental and Dispersal Studies on Vancouver Island

Competing theories have been proposed regarding the origin of *C. gattii* on Vancouver Island (eg, recent introduction, long-term colonization, specific imported vectors). Suffice it to say, the colonization pattern and dispersal of the organism argues against a one-time introduction to Vancouver Island, particularly if the timeline extends only to the first animal and human cases (1998–1999). The first systematic sampling performed on Vancouver Island in 2002 mapped the colonization of *C. gattii* along a 200 km north-south and a 40 km east-west corridor. This study revealed that *C. gattii* is not homogeneously spread in the environment, with central Vancouver Island having a higher percentage of colonized trees and higher concentration of the organism in soil. The heterogeneous pockets of colonization could explain why limited-sampling strategies may miss the organism. Additionally, even though *C. gattii* has been found to be permanently colonized in some areas, it appears to be transiently colonized in others. The permanently colonized sites have yielded *C. gattii* repeatedly over the last 5 years, although transiently positive results may be due to limits of detection or failure of the organism to establish true colonization (Kidd et al., 2007a++). As well, sites that initially appeared to be negative for *C. gattii* have more recently yielded positive environmental samples (Bartlett, unpublished data). It has been shown that in addition to the airborne spread of propagules, wood products,

soil, water, vehicles, and shoes can act as dispersal mechanisms for the organism (Kidd et al., 2007a++). These mechanisms are consistent with the findings of a veterinary case-control study, where statistically significant risk factors for disease in cats and dogs related to soil disturbance within 10 km of cases, logging within 10 km, travel to Vancouver Island, or owner hiking within 6 months of diagnosis (Duncan et al., 2006c). Although limited environmental sampling in the San Juan Islands, Olympic Peninsula, and Oregon has not yielded *C. gattii* (Fraser et al., 2006; Kidd et al., 2007b++; Upton et al., 2007+). Kidd et al. (2007a++,2007b++) reported finding positive environmental samples from islands in the Georgia Strait and in northern Washington.

A rather surprising finding was that co-isolated *C. gattii* strains are heterogeneous. The first isolates distributed to the research community were mostly from one sampling site (central Vancouver Island) and may have unduly influenced our thinking about the composition of the BC outbreak strains (Kidd et al., 2004++; Fraser et al., 2005+; Fraser et al., 2003). In the initial analysis of the *C. gattii* isolates from this site, Kidd et al. (Kidd et al., 2004++) used polymerase chain reaction (PCR)-fingerprinting to demonstrate that 5% represented the VGI molecular type and 95% belonged to VGII (90% of these were VGIIa and 10% were VGIIb based upon a one polymorphic band in the PCR-fingerprint profiles). Subsequent work revealed that the composition of the *C. gattii* population varies in different regions where detailed molecular subtyping of isolates has been undertaken. In the southern extreme of Vancouver Island, VGIIa accounts for 91% of the isolates and the remainder are VGIIb, whereas at another site VGIIa accounts for only 66% of the isolates, with VGIIb and VGI at 19% and 15%, respectively (Bartlett and Kidd, unpublished data). Of course, the genotype frequencies are likely to be dynamic, and repeated sampling is important. Also, additional diagnostic tools sensitive enough to detect and differentiate isolates directly in environmental samples (eg, PCR on soil samples) would facilitate a better understanding of the population structure and mechanisms of spread of the organism. Already heightened awareness of changing ecologic niches has resulted in an expansion of knowledge of the environmental origins of other cryptococcal species (Filion et al., 2006).

Molecular Characterization of Isolates from BC and the Pacific Northwest

Following the initial analyses of genotype frequency described above, Kidd et al. (2005+) used multilocus sequence typing (MLST) and gene genealogy analyses with four genes to examine patterns of molecular variation as well as population structure of the isolates from Vancouver Island compared with a worldwide sample of *C. gattii* strains. This work demonstrated that the VGIIa and VGIIb genotypes originally established by PCR-fingerprinting (Kidd et al., 2004++) corresponded to specific MLST profiles. Similar MLST results with additional genes were obtained by Fraser et al. (Fraser et al., 2005+). Of specific

interest from these studies was the identification of isolates from other areas of the world with identical or similar genotypes to the VGIIa (as represented by isolate A1MR265) and VGIIb (represented by isolate A1MR272) strains from Vancouver Island. For example, the VGIIa genotype was also shared by the NIH444 strain (from a patient in Seattle, ca 1971), CBS7750 (from a Eucalyptus tree in San Francisco, ca 1990) and with isolates from other parts of North America (KB10455 and KB9944) (Fraser et al., 2005+; Kidd et al., 2005+). A Brazilian clinical isolate, ICB107, differed from the VGIIa genotype at only one of 22 loci (Fraser et al., 2005+). The VGIIb genotype was also observed among environmental isolates from Australia (eg, Ram002, Ram005, WM1008), clinical isolates from Australia (eg, NT-6, NT-13), as well as a clinical isolate from Thailand (MC-S-115) (Fraser et al., 2005+; Kidd et al., 2005+). A Caribbean strain 99/473 of the VGIIb type was also found to differ at only one of 22 loci (Fraser et al., 2005+). Intriguingly, two isolates from human cases in Oregon (2004) were recently found to represent subtypes within the VGII genotype that have not identified among any other strains to date (MacDougall et al., 2007++).

The VGIIa and VGIIb isolates from Vancouver Island have been obtained from both clinical and environmental sources. However, the situation is more complex for strains of the VGI genotype from clinical and environmental sources. Specifically, Kidd et al. (2005+) characterized six VGI isolates from Vancouver Island and identified four different genotypes by MLST analysis. Two of these were environmental isolates with a different genotype from the clinical isolates. Thus, in contrast to the VGII types, it was not possible to establish an epidemiologic link between environmental and clinical isolates of the VGI type. However, recent analysis of further environmental VGI isolates from Vancouver Island indicated that they were highly similar to a porpoise isolate (A1MF2863), being identical at four MLST loci (Kidd and Bartlett, unpublished data). It is possible that the clinical isolates of the VGI type represent strains acquired during travel outside of Vancouver Island.

Overall, Kidd et al. (2005+) found that the Vancouver Island isolates were part of a predominately clonal population with little evidence of sexual recombination occurring between them. Fraser et al. (2005+) also presented evidence that the VGIIa and VGIIb strains from Vancouver Island were related in that they shared 14 identical loci out of the 30 examined and proposed that the genotypes represent either siblings arising from a past mating event, or that one may be the parent of the other, perhaps as the result of same-sex mating between *MAT α* parents. Selected isolates from Vancouver Island and other parts of the world have been tested for mating competence. These studies revealed that the VGII isolates are generally fertile whereas VGI strains are not (Campbell et al., 2005; Fraser et al., 2003; Kidd et al., 2004++). In general, the ability of *C. gattii* isolates to mate has implications for recombination events that might generate strains with different virulence properties and environmental adaptability.

The Global Distribution of *C. gattii*

Prior to the emergence of *C. gattii* on Vancouver Island, it was commonly accepted that this species was restricted to tropical and subtropical regions of the world, and that infection was associated with exposure to Eucalyptus trees (Ellis and Pfeiffer, 1990; Kwon-Chung and Bennett, 1984; Sorrell et al., 1996). The idea of a limited geographic distribution came from a study that surveyed a worldwide collection of clinical isolates (Kwon-Chung and Bennett, 1984). This survey revealed that *C. gattii* was prevalent only in regions with tropical and subtropical climates (22%–50% of isolates) relative to *C. neoformans* (50%–71% of isolates). However, this study also reported that 13% of the strains from North America, and 3.3% of the strains from Europe were *C. gattii* (without reference to travel histories). More recent surveys have focused on identifying the molecular types of *C. gattii* found in collections from various regions. In this regard, VGI appears to be the most widely distributed type worldwide (Kidd, 2003; Meyer et al., 2003), and this type is also found most frequently among clinical and environmental isolates in Australia (Campbell et al., 2005). Strains of the VGII type are also found in parts of Australia as well as in North and South America (Fraser et al., 2005+; Kidd, 2003; Kidd et al., 2004++; Kidd et al., 2005+; Meyer et al., 2003). In a recent, large-scale study of IberoAmerican isolates, VGIII predominated, and this type has also been found in India and the United States (Kidd, 2003; Meyer et al., 2003). The VGIV type has been found in Central America and South Africa (Kidd, 2003; Meyer et al., 2003). Notably, the VGIII and VGIV types were not found in the collections from Vancouver Island suggesting that these genotypes may have a more limited distribution.

More recently, Meyer et al. (2007) have surveyed 160 VGII strains recovered globally since 1986 using PCR-fingerprinting, amplified fragment length polymorphism analysis and MLST with eight loci. This work revealed that the VGIIa genotype from Vancouver Island is also found among Brazilian isolates and that Colombian isolates are closely related. Interestingly, the majority of the latter isolates are mating type **a** in contrast to mating type α for the Vancouver Island strains (Escandon et al., 2006), and mating was demonstrated between the Colombian *MATa* strains and VGIIa *MAT α* strains from Brazil and Vancouver Island. This work suggests that the VGIIa genotype was present in South America as early as 1986 and it sheds additional light on the potential mating interactions for VGII types of *C. gattii* that may be relevant for the situation on Vancouver Island.

Overall, these surveys provide an interesting view that the genotypes of *C. gattii* (at least for VGI and VGII) are likely to have a worldwide distribution and the concomitant potential for permanent colonization of suitable environments. This view highlights the need for more extensive environmental sampling globally to generate a detailed picture of genotype frequency over time and location. The most extensive view is now available from the work on Vancouver Island and the lessons learned from this work can be applied in other locations (Kidd

et al., 2007a++), especially with regard to the need for extensive multisource sampling over many years. The wide distribution of *C. gattii* genotypes should also be considered in light of recent reports that infections with this species are occurring in patients with AIDS (South Africa [Morgan et al., 2006], Southern California [Chaturvedi et al., 2005a]). Therefore, it will be important to identify the endemic areas for specific *C. gattii* genotypes in order to monitor human and animal disease.

Origin of the *C. gattii* in BC and the Pacific Northwest: Aboriginal Species or Landed Immigrant?

It is fun to speculate about the origin of the genotypes on Vancouver Island, and this activity has consumed much energy in the research community. However, the extent of global strain dispersal has been demonstrated to be significant (Kidd et al., 2005+, Xu et al., 2000), making it difficult to accurately determine a specific origin of any given genotype. It is possible that the species has been a long-term resident of BC and that changing conditions (eg, climate or land use) or improved surveillance are responsible for the current level of awareness. Alternatively, it has been suggested that the emergence is due to the recent introduction of a particularly virulent genotype that may be well adapted to the local conditions such that large numbers of infectious cells are propagated (Fraser et al., 2005+). Although it may be difficult to garner strong evidence for a given theory, it is clear that much more information is needed about the *C. gattii* genotypes on Vancouver Island and worldwide and about the disease caused by *C. gattii* in immunocompetent hosts. Below, we discuss some of the studies that are needed to generate a more detailed view of *C. gattii* that may help in infection control.

Ecologic adaptability, colonization, and dispersal

The environmental sampling revealed a high level of soil colonization on Vancouver Island, and it would be interesting to examine soil persistence and competition in laboratory and field settings. These types of experiments may be relevant to addressing how the fungus becomes aerosolized and the nature of the infectious particle. An investigation of conditions required for the propagation of the infectious particles in soil/trees would also be highly relevant to understanding the factors that influence exposure of humans/animals.

It is likely that no one factor can explain the dramatic emergence of *C. gattii* on Vancouver Island, and there may be interplay between soil conditions, temperature, and moisture. Current weather station data are insufficient to adequately describe the microclimates in areas colonized by the pathogen. Climate oscillations driven by alternating El Niño and La Niña currents have produced both drier and wetter than normal summer conditions in BC over the last few decades. Outbreaks of another fungal disease, coccidioidomycosis, have been

shown to follow soil disruption in California (Zender and Talamantes, 2006). Data gathered from the BC environment conclusively show that *C. gattii* is well adapted to survive in dry, low nutrient soil and is more likely to be airborne during dry summer weather (Kidd et al., 2007a++). The stability of the colonization of soil and trees at permanently colonized sites suggests that the pathogen can effectively compete with resident soil microflora. Longer cycles of meteorology patterns and finer tools of climate measurement will be needed to understand the complex relationship of microbe, climate, and ecologic niche.

Additional sampling around the world is needed to investigate predicted favorable climate/soil/water conditions that might allow colonization by *C. gattii*. Mak (2007) has recently developed ecologic niche models that predict the probable extent of environmental colonization of *C. gattii* based on human, animal, and environmental data and climate projections for the Pacific Northwest (Fig. A1-1). Areas that may eventually be impacted include the Lower Mainland of BC with a population base of approximately 2 million people. These projections could be used by public health officials on both sides of the US-Canada border to plan strategies for risk communication and anticipated morbidity and mortality (Mak, 2007).

Clinical considerations

Perhaps the most relevant topics regarding the emergence of *C. gattii* have to do with identifying risk factors for people, designing ways to limit exposure, and developing effective methods to treat the infections that do occur. It is common to see statements in the literature that *C. gattii* is a primary pathogen that infects immunocompetent people, and that *C. neoformans* is an opportunistic pathogen that infects immunocompromised people. The distinction may be less clear given that *C. gattii* is now being found in AIDS patients and *C. neoformans* can infect seemingly immunocompetent people (Chaturvedi et al., 2005a; Morgan et al., 2006; Speed and Dunt, 1995). There is clearly a need for retrospective studies of patients to determine host risk factors as well as prospective case studies to determine efficacy of treatments. The number of cases continuing to occur on Vancouver Island (and among tourists [Lindberg et al., 2007]) would allow this type of investigation.

An interesting consideration in terms of exploring possible virulence differences for *C. gattii* versus *C. neoformans* is whether mouse virulence studies have relevance for human disease. For example, the strains with the VGIIa and VGIIb genotypes from Vancouver Island both cause disease in humans, but laboratory studies revealed virulence differences between the two strains tested (Fraser et al., 2005+). The more virulent strain, A1MR265, of the VGIIa genotype showed equal virulence in the mouse model to strain H99 that is representative of the most common VNI type of *C. neoformans* (var *grubii*). It is possible that these results reflect the fact that only one isolate of each genotype from Vancouver Island

was tested and the isolates selected may not be representative. It is clear, however, that strains of *C. gattii* show virulence differences (Kronstad, unpublished data) (Chaturvedi et al., 2005b; Fraser et al., 2005+) and that multiple isolates from Vancouver Island and worldwide collections need to be tested. The same is true for *C. neoformans* as demonstrated by the range of virulence detected by Clancy et al. (2006). Thus, we need to develop better models to assess differences in virulence and to explore possible differences that may be relevant to infection of immunocompetent versus immunocompromised hosts.

Applications of genomic approaches to develop a detailed understanding of C. gattii

The emergence of *C. gattii* provided the impetus to sequence the genomes of isolates representing the VGI (WM276) and VGIIa (A1MR265) genotypes (Michael Smith Genome Sciences Center, 2007++; The Broad Institute, 2007++). These are important resources for the next steps in characterizing the virulence of *C. gattii*, the genetic diversity of the species and the interactions of the fungus with the environment. One can imagine, for example, using the genomes for transcriptome and proteome studies to identify differences in expression for *C. gattii* relative to *C. neoformans*. Some of these differences may reveal factors that contribute to the primary pathogenesis of *C. gattii* relative to *C. neoformans*. The two *C. gattii* genomes also provide a platform for more detailed analyses of genotypes and comparative studies of genome variability. In the latter case, comparative hybridization or genome resequencing approaches can be used to study the microevolution of genomes in strains in the environment and clinical strains during passage through human and animal hosts (eg, during relapse or drug therapy). Comparative genome hybridization experiments with the VGI and VGIIa genomes have been initiated to identify genomic changes in mutants that have lost virulence and to examine genome variation in strains representing the VGI, VGIIa, and VGIIb genotypes (Kronstad, unpublished data). The declining cost of sequencing will also allow further genome-sequencing projects to provide a deeper view of genome content and variability. The more detailed information may eventually lead to the separation of the molecular types of *C. gattii* into distinct varieties or species.

Media Coverage of the Emergence of *C. gattii*

Any emerging infectious disease represents a challenge to the public health system. The system must respond to educate caregivers about appropriate interventions while balancing the message to allow the public to make informed choices. For example, the lay press recently reported concern by members of the public in Alabama where experimental plots of genetically engineered Eucalyptus trees will be grown; the fear being that *C. gattii* will be imported into the environ-

ment through the Eucalypts (United Press International, 2007), even though no link to Eucalyptus was shown in the BC experience (Kidd et al., 2007a++). In an examination of press coverage of *C. gattii* as an emerging infectious disease agent, researchers at the University of BC Centre for Health and Environment Research found that during the period 2001 to 2006, BC newspapers carried 422 articles warning the public about West Nile Virus (although no West Nile Virus cases have been reported in BC) compared with 79 articles about *C. gattii* (170 human cases, eight deaths) (Nicol et al., unpublished data). The research group concluded that because West Nile Virus is a public health risk with identifiable precautionary actions in central Canada, newspapers were more likely to print stock West Nile Virus stories. *C. gattii* was seen to be a local phenomenon with no identifiable risk aversion strategies and to have potential economic repercussions to the areas affected and so was less reported. There also seemed to be confusion by news writers about the biology of *Cryptococcus* because the term “virus” seems to be better understood as a pathogen compared to “yeast” (Nicol et al., unpublished data). Similarly, some news items labeled *C. gattii* as an “Australian” fungus despite the body of literature cited above on the global distribution of the pathogen. Overall, these observations demonstrate that effective education of the media and the public is a critical component of the management of an emerging infectious disease.

Conclusions

A great deal has been learned about the emergence of *C. gattii* in BC over the past 8 years. We now have a clear picture of the environmental sources of the pathogen and mechanisms of dispersal, we have an understanding of the genotypes that are causing disease in humans and animals, and we have some information about clinical presentation and treatment. Certainly, there is a great deal more to investigate in terms of risk factors for the human population and treatment outcomes. In this regard, the situation on Vancouver Island presents an opportunity to develop a detailed view of an emerging infectious disease with regard to environmental exposure, the role of sentinel animals in monitoring risk, and the underlying factors that influence human susceptibility. This information may prove useful for other emerging diseases and provide methods to manage both the ongoing situation in BC and the apparent emergence of the disease in the Pacific Northwest.

Acknowledgments

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A2

THE GOOD, THE BAD, AND THE UGLY: FUNGI MOLD YOUR WORLD

*Meredith Blackwell*³

Fungi⁴ are important members of many ecosystems. As heterotrophs they are involved in nutrient cycles, especially of carbon, nitrogen, and phosphorus. The effects of fungi were observed in prehistoric times, and their part in causing plant disease was understood before the germ theory was advanced. Today fungi are featured in the popular press and science Internet postings, indicating that they are of increasing interest and importance. Molecular methods have helped to popularize fungi by bringing rapid progress to fungal classification and discovery and have enhanced understanding of their biology. Fungi are associates of all major groups of organisms and are especially well known for their interactions with plants and insects. Fungi also are economically important and provide drugs, foods, and fermented beverages. The value of fungal activities and products far exceeds the costs of the diseases they cause.

Introduction

Human beings were aware of fungal fruiting bodies in prehistoric times, and the sudden appearance of mushrooms after rain awed those who did not comprehend the fungus lifecycle. Lowy (1974) wrote that the sudden appearance of mushrooms of *Amanita muscaria* was believed to have been caused by thunder-

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⁴ In addition to members of Kingdom Fungi, several other organisms of the fungus-like group Oomycota (*Phytophthora*) are included.

bolts as they struck the ground, a belief held independently in Roman, Hindu, and Mayan cultures. Humans endowed mushrooms with magical properties (Wasson, 1968), and evidence of early fungal use exists in many parts of the world. Grave guardians, masks, clothing ornaments, and other artifacts were made from the fruiting bodies of wood-decaying basidiomycetes such as *Fomitopsis officinalis* and *Haploporus odoratus* (Blanchette, 1997; Blanchette et al., 1992a, 2002). A surviving mushroom culture centered on magic mushrooms existed in Oaxaca for many years, and the celebrated *curandera*, Maria Sabina, was visited by a number of prominent individuals and notable musicians who sought her spiritual guidance (Wasson, 1957, 1976). Although yeasts themselves were not known, evidence of their activity comes from residues in nine millennia-old Neolithic vessels (Vouillamoz et al., 2006).

Plant pathogenic fungi also were known in ancient times. Three centuries BC, Theophrastus recognized fungi as the cause of certain diseases of crops, but by the first century, the knowledge had been lost, and Pliny attributed lost yields to the gods or stars (Carefoot and Sprott, 1969). Fungal effects such as disease were not understood by many until the observations and experiments of Miles Joseph Berkeley and Anton de Bary around the time of the Irish potato famine of 1845–1846. This work actually came before the general acceptance of the germ theory. The contribution of de Bary also argued strongly against a lingering belief in spontaneous generation (Matta, 2010). Fungi continue to appear suddenly as they invade natural landscapes to cause diseases of plants and animals. The invading organisms often are not noticed until they encounter naïve hosts in new regions where they cause devastating diseases. Earlier invasions included the chestnut blight fungus and several waves of Dutch elm disease fungi (Alexopoulos et al., 1996). The papers in this volume, *Fungal Diseases: An Emerging Threat to Human, Animal, and Plant Health* (IOM, 2011), cover the newest waves of invasive fungal diseases and their attack on naïve hosts.

More important, however, is the realization that fungi are essential for life on Earth. Fungi are decomposers that destroy plant and animal bodies and return carbon, nitrogen, phosphorus, and other minerals to nutrient cycles. Compatible with their primary role in decomposition, fungi interact with other living organisms in nutritional relationships, and their secondary metabolites and enzymes supply medicines, food and drink, and industrial products for profitable enterprises. Fungi appear regularly in newspapers and magazines. Over the past year, the *New York Times* featured fungi prominently. Articles have included reports of the identification of a microsporidian fungus partly responsible for colony collapse disorder of bees, a chytrid responsible for global amphibian decline, *Geomyces destructans* of bats, and pathogens of home garden vegetables. Ecological topics included interactions between bark beetles and fungal symbionts, mycorrhizal associations, sexual reproduction in truffles, a fungus that exerts selective pressure on rotifers, and fungal function in the environment. Fungi also have been covered in the *Wall Street Journal* as food items, inhabitants of saunas, and the “Torula

of Cognac,” *Baudoinia compniacensis*, the fungus that grows on walls of wine cellars in mists of alcoholic vapors. One fungus was reported widely because it prompted a murder investigation in a German forest when its sulfurous odor of decay was mistaken for that of a dead body (Anonymous, 2005). Coverage of a broad range of fungal topics also can be found in science blogs and Internet postings with reports of jet lag expressed in circadian rhythms of fungi; wood decay; the evolutionary arms race between a smut fungus and maize; a new species of introduced, beetle-associated fungus that kills plants in the Lauraceae; and yeast genome sequencing leading to improved bioethanol production. *National Geographic News* also reviews interesting fungal topics, including stories on endophyte biology and “bringing order to the fungus among us,” describing the Assembling the Fungal Tree of Life project (see below). Only 2 days before this meeting (December 12, 2010), *USA Today* published an article by Elizabeth Weise, “Why it’s cool to have a fungus among us.” The informative article could have been the basis for this talk—if only it had appeared earlier. The range of examples cited indicates a growing interest in and knowledge of fungi.

Fungi influence our daily lives in ways we seldom appreciate. Several entrenched fungal-influenced cultural practices are the result of fungal plant diseases. These include tea drinking in the United Kingdom, a switch imposed by devastation of coffee plants in Ceylon (present-day Sri Lanka) by the coffee rust fungus (*Hemileia vastatrix*) in the late 1800s (Horsfall and Cowling, 1978); consumption of cornbread as a staple in the southern United States colonies was imposed because the wheat rust fungus prevented wheat cultivation in the humid South (Horsfall, 1958); and the enjoyment of gingerbread comes from the time when the effects of stinking smut of wheat were masked by molasses and ginger (Carefoot and Sprott, 1969). We rely on fungi for clothing fads such as use of cellulase enzymes of species of *Trichoderma* to speed the “stone washing” of our blue jeans (Bhat, 2000). Perhaps, fungi may make us more beautiful when certain “integrative approaches to better skin”⁵ are followed using a blend of fungi that includes *Cordyceps*, reishi mushrooms, and other ingredients.

The Classification and Discovery of New Species of Fungi

Early phylogenetic studies based on DNA sequences defined a monophyletic⁶ group of Fungi. Oomycota and relatives, various slime mold clades, and several other groups previously considered as zygomycetes have been excluded from the monophyletic Fungi. Asexual and sexual fungi could be combined on the basis of their genetic relationships, and asexual groupings of asexual fungi were abandoned (Alexopoulos et al., 1996). More recently, mycologists have increased

⁵ Several brands of skin creams include a variety of basidiomycete fruiting bodies as ingredients that are said to provide for skin relief and other effects (e.g., Dr. Weil’s Mega-Mushroom lotions, cleansers, and serums).

⁶ A group of taxa containing an ancestor and all its descendants.

the number of DNA markers and taxa in diverse clades to produce increasingly well-resolved phylogenies,⁷ the basis of predictive classifications (Figure A2-1) (Hibbett et al., 2007; James et al., 2006; White et al., 2006). An issue of the journal *Mycologia* (98:829–1103, 2006) was devoted to the phylogenetics of many major groups of fungi. Recent phylogenetic studies have provided new insights into fungal relationships and show that the earliest diverging lineages of zoosporic⁸ and zygosporic⁹ groups are not monophyletic as previously assumed on the basis of morphological characters and that they are more diverse than previously understood. Other findings provide data to include microsporidia within or very near the fungi (Lee et al., 2010). The new phylogenetic studies are largely the result of several National Science Foundation projects (Research Coordination Networks: A Phylogeny for Kingdom Fungi [Deep Hypha] and Assembling the Fungal Tree of Life 1 and 2) that involved more than 100 biologists from about 20 countries (Blackwell et al., 2006; Hibbett et al., 2007). Current projects under way include adding taxa to expand the fungal tree of life and pursuing an increasing number of genomics projects.

About 100,000 species of fungi have been described, but a conservative estimate suggests that there are 1.5 million fungi on Earth (Hawksworth, 1991, 2001). The estimate has spurred exploration for the million fungi that remain undiscovered (Figures A2-2A through A2-2D).

More recently the 1.5 million estimate was surpassed by a higher estimate of 3.1 to 5.1 million species based on the use of molecular methods, including high-throughput sequencing (O'Brien et al., 2005). Because of the great discrepancy between known and estimated fungal species numbers, mycologists have a renewed interest in fungal discovery. Many have wondered, where are the missing fungi (Hyde, 2001)? If the higher estimates are realistic, the number of fungi is equal to the number of animal species and may exceed the number of plants by 10:1. Abundant evidence shows that many tropical fungi remain to be discovered based on species accumulation curves of fungi collected in plots (Aime et al., 2010). Other habitats reporting large numbers of fungi include living leaves of tropical trees (Arnold, 2007), soil fungi (O'Brien et al., 2005; Taylor et al., 2010), and even the fungi in the buildings in which we spend most of our time (Amend et al., 2010). Many fungi, however, remain to be discovered in northern temperate regions, including far northeastern Asia (Petersen and Hughes, 2007). We do not have to look for undescribed fungi in completely new places or tropical regions, however, because they may be in our backyards. My colleagues and I look for new species among the yeasts and other microscopic fungi that are difficult to see

⁷ A phylogeny is an inferred history of evolutionary relationships of organisms; often depicted in a tree diagram.

⁸ Zoospores are flagellated cells of certain fungi (see Figure A2-1) that are produced in sporangia in asexual reproduction.

⁹ Zygosporangia are thick-walled spores produced in some fungi (see Figure A2-1) resulting from the fusion of like gametes.

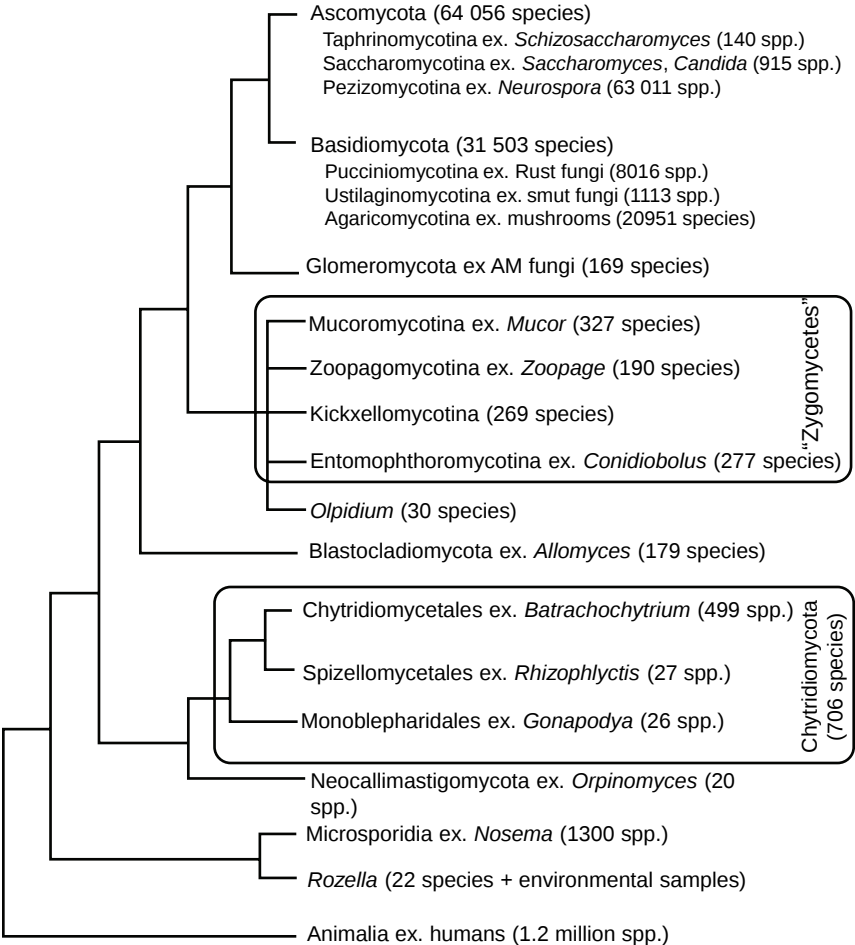


FIGURE A2-1 Diagrammatic representation of relationships of fungal taxa, examples (ex.), and approximate number of species in each group. Zoosporic and zygosporic fungi are more diverse than previously recognized on the basis of morphological traits, and they are not monophyletic. Two flagellated taxa, *Rozella* and *Olpidium*, are of uncertain taxonomic placement. Evidence from multilocus sequencing and genomics reveals that microsporidians branch within or near fungi. Ascomycota and Basidiomycota, the most speciose phyla, are each divided into three subphyla. The largest number of fungal species are classified in the subphyla Pezizomycotina and Agaricomycotina.

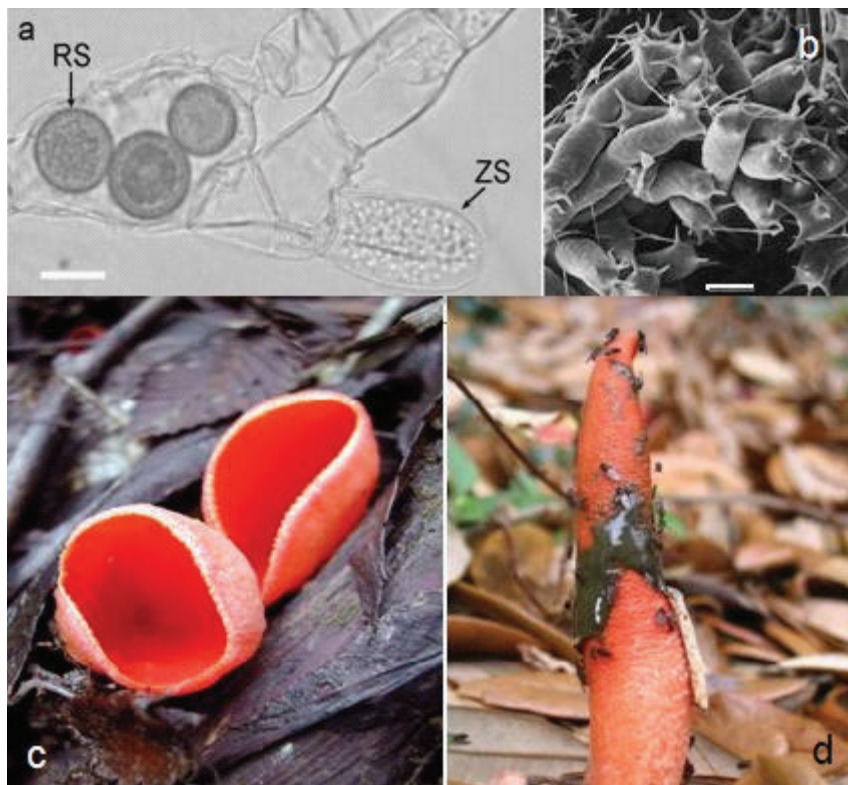


FIGURE A2-2 Images of representative fungal groups. (A) Hyphae of the blastocladiacean fungus, *Allomyces* sp. Note a terminal zoosporangium (ZS) containing zoospores. The spiny, dark, thick-walled resting spores (RS) within the hyphae are those of a zoosporic fungal parasite, *Rozella allomycis*, of uncertain taxonomic placement. Bar = 10 μ m. (B) *Lobosporangium transversale*. The zygosporic fungus in the Mortierellales has unusual spiny lobed sporangia (Benny and Blackwell, 2004). Bar = 50 μ m. (C) *Sarcoscypha coccinea*. The several cm diameter fruiting body of the scarlet cup ascomycete, *Sarcoscypha coccinea*. Ascospores are formed within asci on the inner surface of the cup. (D) *Mutinus* sp. A stinkhorn similar to one mentioned in the text that caused a search for a dead body (Anonymous, 2005). Stinkhorns produce noxious compounds that attract insect spore dispersers. The dark slimy mass of spores has been partially removed by flies.

SOURCES: (A) photo courtesy of Timothy Y. James, provided by Meredith Blackwell (2009). (B) micrograph courtesy of Kerry O'Donnell, provided by Meredith Blackwell (2004). (C) photo courtesy of eriotropus/coqui, provided by Meredith Blackwell (2002). (D) photo courtesy of Nhu H. Nguyen, provided by Meredith Blackwell (2005).

with the unaided eye (Boekhout, 2005; Suh et al., 2005), and members of early diverging lineages that often are difficult to isolate and culture. Ascomycetes and basidiomycetes are expected to provide the greatest diversity of additional taxa based on numbers of currently known fungi, but certainly the developing methods using high-throughput sequencing of DNA will lead to the discovery of more of the early diverging groups (Figure A2-1) (Kirk et al., 2008).

Examples of large numbers of species isolated into culture from certain substrates include the finding of 418 unique morphotypes of endophytic fungi from 83 leaves in Panama (Arnold, 2007), 257 fungal endophyte genotypes in coffee plants (Vega et al., 2010), and 650 yeast isolates representing 290 genotypes of nearly 200 undescribed taxa from the gut of beetles (Suh et al., 2005). Acquiring cultures and specimens will remain important in cases when fungi and cultures are needed for certain purposes, including population studies, environmental remediation, and secondary metabolites. Taylor and his colleagues (2010) used high-throughput sequencing to estimate the presence of more than 200 taxa in a 0.25 g soil sample with only 14 percent overlap in taxa in a sample taken a meter away. If we are to determine the number of fungi on Earth, environmental sequencing will be necessary to speed fungal exploration and discovery. In addition to new species, entire lineages, some probably at the level of subphylum, may be recognized by DNA sequences such as Soil Clone Group 1 (Porter et al., 2008; Rosling et al., 2010). More work will be needed to determine geographical and substrate ranges in order to obtain more accurate estimates of species numbers.

Species discovery is relevant to the topic of this workshop because previously unknown plant and animal pathogenic fungi have been introduced into the United States many times. These fungi probably caused few symptoms and went unnoticed in their native hosts. Devastation of naïve hosts, however, led to their recognition and subsequent description as new species. This scenario certainly is repeated by the fungi discussed in this meeting, including *Batrachochytrium dendrobatidis*, the pathogenic chytrid of amphibians spread around the world; *Geomyces destructans*, the pathogen of bats in North America; and *Phytophthora ramorum*, causing declines of certain plants in North America and Europe. Prior invasions have included several fungal agents of Dutch elm disease; the chestnut blight fungi; the newly arrived agent of the laurel wilt delivered within the mycangia¹⁰ of its ambrosia beetle vector; and *Discula destructans*, a pathogen of North American dogwoods (Alexopoulos et al., 1996; Harrington and Fraedrich, 2010; Zhang and Blackwell, 2001). Recently, a new approach to discovering the native ranges of certain fungi has been profitable. Ning Zhang (Personal communication, Rutgers University, December 10, 2010) designed an efficient assay method using specific primers to detect the dogwood pathogenic fungus in herbarium specimens. The method promises to greatly reduce the time involved in determining geographical and host ranges and is ideal for working with col-

¹⁰ Mycangia are pouch-like invaginations in the cuticle of certain insects used to transport cells and spores of symbiotic fungi, found especially in some species of bark and ambrosia beetles as well as a few other groups of insects.

laborators at herbaria throughout the world. Because patterns of introduction of pathogens may exist, determination of native ranges is essential in combating invasive organisms.

Supermodels

Fungi are important as model systems in research. *Saccharomyces cerevisiae* (Figure A2-3) is a supermodel known for its baking and brewing prowess and as the first eukaryote to have its entire genome sequenced.

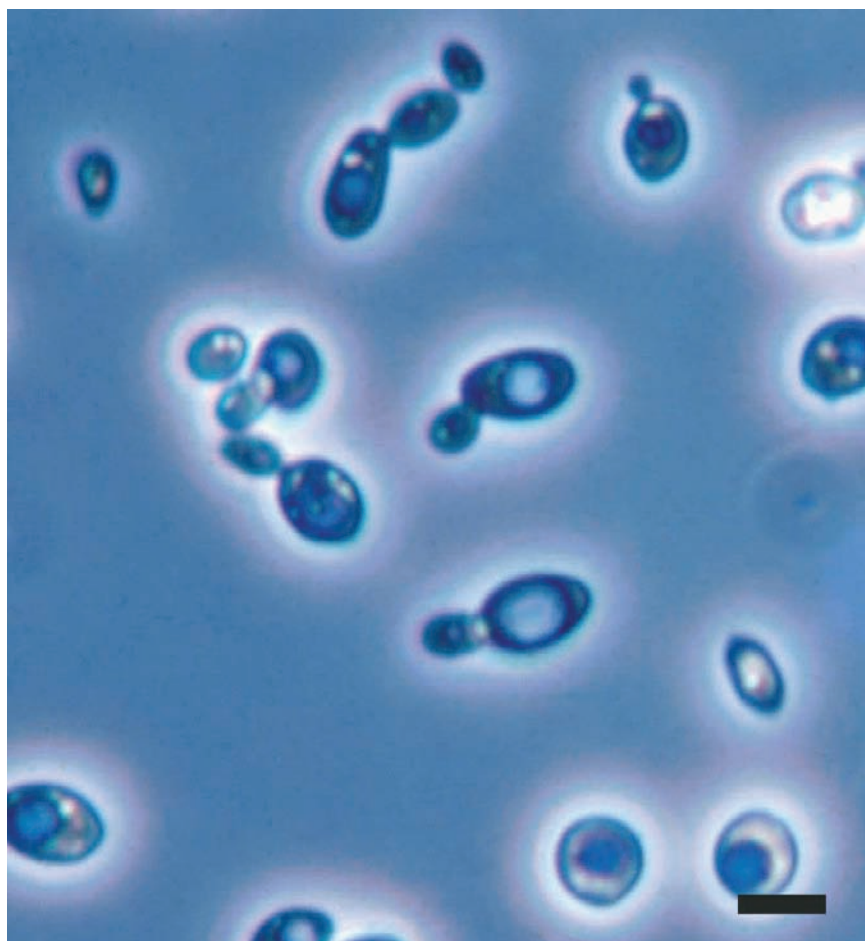


FIGURE A2-3 *Saccharomyces cerevisiae* (Y-2235), baker's yeast and model organism. Note the many budding cells in the stained preparation. Bar = 5 μm .
SOURCE: Photo courtesy of Cletus P. Kurtzman, provided by Meredith Blackwell (2008).

In addition to *S. cerevisiae*, three other fungi that have been important in research and were the subjects of Nobel Prize-winning research are *Schizosaccharomyces pombe*, another fast-growing organism with a yeast growth form; *Penicillium chrysosporium*, producer of the first effective antibiotic; and *Neurospora crassa*. In his Nobel Prize acceptance speech, Tatum (1958) acknowledged, among others, “B.O. Dodge for his establishment of this Ascomycete as a most suitable organism for genetic studies.” Beadle (1958) also spoke of *Neurospora crassa* and pointed out that “Dodge was an enthusiastic supporter of *Neurospora* as an organism for genetic work. ‘It’s even better than *Drosophila*,’ he insisted to Thomas Hunt Morgan, whose laboratory he often visited. He finally persuaded Morgan to take a collection of *Neurospora* cultures with him from Columbia University to the new Biology Division of the California Institute of Technology, which he established in 1928.” This was the beginning of the development of *Neurospora crassa* in genetics research.

As mentioned above, *S. cerevisiae* was the first eukaryotic organism to have its entire genome sequenced. This yeast and other species in the Saccharomycotina have relatively small genomes that make them economical candidates for sequencing (Mewes et al., 1997). In addition, yeasts and other model fungi are easy to grow and complete their lifecycles in culture in a few days; because they are haploid throughout most of their lifecycle, induced mutations are expressed rapidly. Many fungi, including some yeasts, also have a sexual state from which all products of meiosis can be isolated in addition to asexual spores and somatic cells from which uniform populations can be established. They also are excellent organisms for population studies (Anderson et al., 2010). Some fungi, including *S. cerevisiae*, have morphological cues that indicate the occurrence of certain cell cycle events, and a large body of background information is available for previously established model fungi studies. Improvements in genome sequencing have made it possible to develop many new “models,” including plant and animal pathogens and their hosts. For example, yeasts from the gut of wood-feeding beetles have been of particular interest because many of them ferment xylose, a requirement for efficient digestion of lignocellulose in biofuel production. These species have undergone biochemical and metabolic engineering to obtain more information on xylose fermentation pathways, and genome sequencing is important toward this end (Jeffries et al., 2007; Joint Genome Institute, 2007; Van Vleet and Jeffries, 2009).

Fungi Make Money: Useful Fungal Products

Humans have used a variety of fungal products for different purposes, including cures. In fact some of the magical fungi mentioned above also have been used for their medicinal properties, which may have been known since prehistoric times. Evidence exists for the use of fungi by early humans. Ötzi the Iceman lived about 5,300 years ago, and his mummified body was discovered in 1991

on the border of Italy and Austria. He carried pieces of the fruiting bodies from two species of wood-rotting basidiomycetes, *Piptoporus betulinus* and *Fomes fomentarius*, perhaps for medicinal uses (Peinter et al., 1998). Other writers have suggested that one of the fruiting bodies was used as a strop for sharpening knives and tools, but whatever their use, fungi appear to have been important to Copper Age Europeans.

Some basidiomycetes have been used medicinally in more recent times. Extracts of *Inonotus obliquus* was used in Europe as a treatment for cancer, and the fruiting bodies of *Fomitopsis officinalis* (the quinine conk), mentioned earlier as grave guardians in the Pacific Northwest, were also harvested for medicinal properties. A different kind of medicinal use by foresters was the application of sheets of mycelium on ax injuries to stop bleeding (Gilbertson, 1980). The spore masses of giant puffballs that were discovered stockpiled along Hadrian's Wall (in Northern England) also have been used as a styptic (Personal communication, Roy Watling, former Head of Mycology and Plant Pathology, Royal Botanic Garden Edinburgh, August 27, 1977), and spores of unspecified puffballs also were widely used as a styptic by natives of North America as well (Blackwell, 2004).

Certain ascomycete fungi, previously known as species of *Cordyceps*, have been used in Asian traditional medicine for several centuries (Spatafora et al., 2007). One of these fungi, a parasite of caterpillars, known as *Cordyceps sinensis* since 1878, now is *Ophiocordyceps sinensis* based on a phylogenetic study (Sung et al., 2007). Recent interest in the fungus has provided evidence that it may be effective in the treatment of certain tumors (Spatafora et al., 2007). The revision of the entire group of insect-pathogenic fungi previously placed in the genus *Cordyceps* has resulted in the placement of species in three different families (Sung et al., 2007). This is an important development because phylogenies are predictive of traits common to closely related fungi, and other *Ophiocordyceps* species may be targeted for the mining of metabolites. The efforts to develop penicillin for the treatment of bacterial infections at the beginning of World War II resulted in the discovery of a long-sought magic bullet and hastened the rise of the modern pharmaceutical industry. In addition to the fungus-derived drug penicillin, three statin drugs for lowering cholesterol levels (e.g., Lipitor®) and the immune suppressant cyclosporine each have earned more than a billion dollars annually. Cyclosporine, once critical to transplant surgery, is today used to treat dry eye as well as more serious conditions (Blackwell, 2011).

Fungi also are big business in the food and beverage industries. In addition to the usual fresh fruiting bodies of basidiomycetes (mushrooms) and a few highly favored ascomycetes (truffles and morels), other fungi, such as *cuitlacoche* (corn smut) and rice smut, are eaten in Mexico and Asia, respectively. Processed foods also are made from fungi. These include yeast extract spreads such as marmite and vegemite and the meat substitute, Quorn™, a product of hyphae of an ascomycete, a species of *Fusarium*. Several species of *Aspergillus* are used in the processing of soy sauce, and fungi play a part in the flavoring process of cheeses.

Throughout the world many fermented foods rely on fungi at least in part to increase nutritional value, improve texture and flavor, and preserve the foodstuff. In one short street block in Brussels, I examined shop windows to count the many products that had been touched by fungi: coffee, certain teas, chocolate, cheeses, bread, salami and dry-cured hams, and numerous fermented beverages (Tamang and Fleet, 2009). Many African and Asian foods, including miso, ontjom, and tempeh, are the products of fermentation (Nout, 2009; Rodríguez Couto and Sanromán, 2006).

As in the case of other fungal products, the making of alcoholic beverages almost certainly was discovered millennia ago, found accidentally in prehistoric times when wild yeasts settled into a sugary beverage. Yeasts are essential to the multibillion-dollar alcoholic beverage industry. In the United States, sales of beer, spirits, and wine were \$116 billion in 2003 (Library Index, 2011). The yeasts involved in brewing were first isolated into pure culture by Emil Hansen at the Carlsberg Brewery in Copenhagen, and the brewery lab became an important site of classic yeast genetics and biotechnology research (Hansen and Kielland-Brandt, 2003). Pretorius (2000) suggested that many additional yeast species might be used in winemaking. In this context my colleagues and I have discovered nearly 300 previously unknown yeasts, many of which have the ability to ferment a variety of sugars, yet are untried for making beverages (Suh et al., 2005; Urbina and Blackwell, unpublished). In addition to its significance in brewing and bread making, *S. cerevisiae*, of course, has been extremely important in industrial biotechnology because of the development of efficient transformation methods and specialized expression vectors, and for a variety of other genetics tools (Nevoigt, 2008).

Fungi Interact with Other Organisms

Fungi interact with all major groups of organisms. Specific interactions with photosynthetic organisms are generally well known (Table A2-1). About 80 percent of all plant species and 92 percent of plant families form close associations with fungi known as mycorrhizae (Smith and Read, 2008; Trappe, 1987). Fungi and plant roots or underground stems form several kinds of mycorrhizae that are classified by the morphology of the interacting fungus in relation to the root. The associations are important for carbon, mineral, and water exchange, with carbon generally transferred from the plant to the fungus.

Arbuscular mycorrhizal (AM) fungi are known from the 400 million-year-old Rhynie chert. The fungi penetrate the plant cell wall and form a highly branched arbuscule that invaginates the plasma membrane of the root cortex cells. The 200 members of the asexually reproducing phylum Glomeromycota are obligate fungal partners of about 60 percent of all plant species. Hosts include a variety of crop and forage plants such as maize, rice, alfalfa, and citrus, as well as many non-cultivated plants. Molecular methods have detected previously unknown host

TABLE A2-1 Examples of Fungal Associations with Plants

Association	Plants	Fungus	Reference
AM Mycorrhizae	60% of all species	Glomeromycota	Selosse et al. (2006)
Ectomycorrhizae	2,000 species	~5,000 species of basidiomycetes, ascomycetes, Endogenales	Smith and Read (2008)
Endophytes	95% of all plants	Many groups of ascomycetes and some basidiomycetes	Rodríguez et al. (2009)
Lichens	~100 species of photobionts (green algae, blue/green bacteria)	~32,000 ascomycetes (Leotiales, Dothideales, and Pezizales), a few basidiomycetes	Schoch et al. (2009)

specificity in some cases (Selosse et al., 2006). Ectomycorrhizal fungi (Figure A2-4) are associated with fewer hosts, including certain dominant forest trees such as birch, dipterocarp, eucalyptus, oak, and pine. Greater ectomycorrhizal fungal diversity is evident, and basidiomycetes, ascomycetes, and a few zygomycetes are involved in these associations. Many of the fungi are generalists, but more specificity occurs than among AM associates. The fungi produce an external mantle over young roots and often cause dramatic shortening and dichotomous branching of the mycorrhizal root (Smith and Read, 2008).

Endophytes are fungi that usually grow within above-ground plant parts without causing disease symptoms in about 95 percent of all plants examined (Arnold, 2007). The fungi that form the associations have been placed in four groups, depending on host specificity, tissues colonized, and amount of colonization within the plant (Rodríguez et al., 2009). Hypocrealean endophytes of grasses and sedges produce alkaloids that have been suggested to deter feeding by insects and vertebrates. Endophyte-infected grasses have enhanced growth and drought resistance (Rodríguez et al., 2009). A different group of endophytes is more taxonomically diverse and has broad plant host range with restricted growth within the plant, often occupying only a single cell. Some of these horizontally transmitted endophytes convey protection from plant pathogens (Arnold et al., 2003; Rodríguez et al., 2009). An endophyte was reported to convey heat tolerance to its grass host near a hot springs in Yellowstone National Park, but additional research has shown that only virus-infected endophytes convey thermal tolerance, a sign of the complexity of such associations (Márquez et al., 2007).

About half of the estimated 64,000 ascomycetes (e.g., Leotiales, Dothideales, and Pezizales) and a few basidiomycetes are the fungal associates (mycobionts) of about 100 species of photosynthetic organisms (photobionts) to form lichens

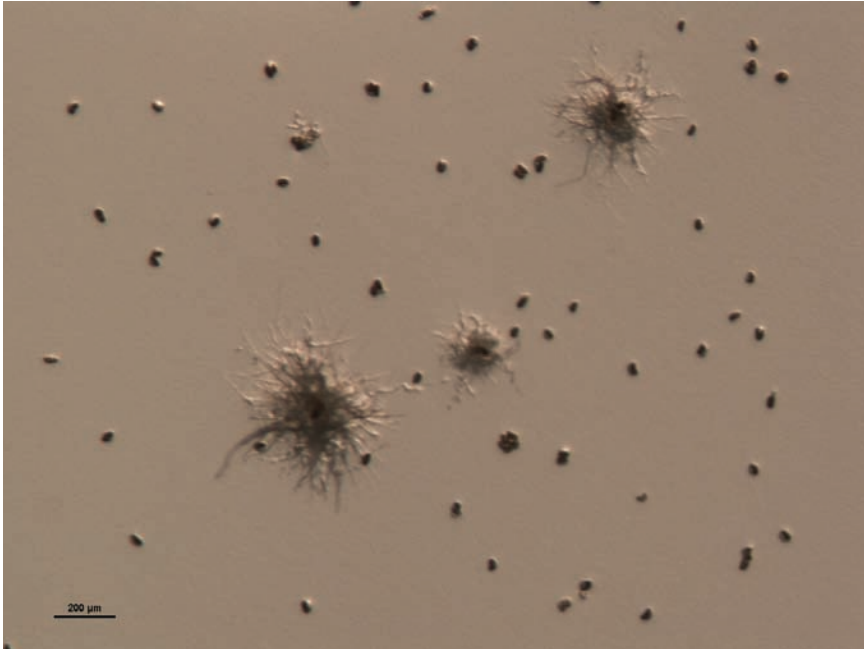


FIGURE A2-4 *Anaptychia ciliaris*. Small colonies of the lichen-forming fungus on agar medium after 3 months of growth. Bar = 200 µm.

SOURCE: Photo courtesy of Ning Zhang, provided by Meredith Blackwell (2010).

(Schoch et al., 2009). Lichens have been used as indicators of pollution. In addition to the photosynthetic partner, usually a green alga, a photosynthetic, nitrogen-fixing blue/green bacterium also may occur in a tripartite association in the lichen. Although the fungal associate can be grown on artificial media, they usually grow very slowly (Figure A2-5). Lichens are hosts for pathogenic fungi as well as endolichenic fungi, the lichen equivalent of endophytes. Each partner in the lichen has a scientific name, but the name of the lichen as a whole is that of the fungus (Ahmadjian, 1993; Nash, 2008).

Wood-Decaying Fungi

Fungi are heterotrophic and their ability to degrade organic materials and return them to nutrient cycles is an essential activity in almost all ecosystems. The ability of a fungus to degrade specific substrates depends on the enzymes it produces, and certain fungi are especially important in forest ecosystems where they are the primary decomposers of wood. Basidiomycetes and some ascomycetes are the primary decomposers of plant cell wall carbohydrates (cellulose and

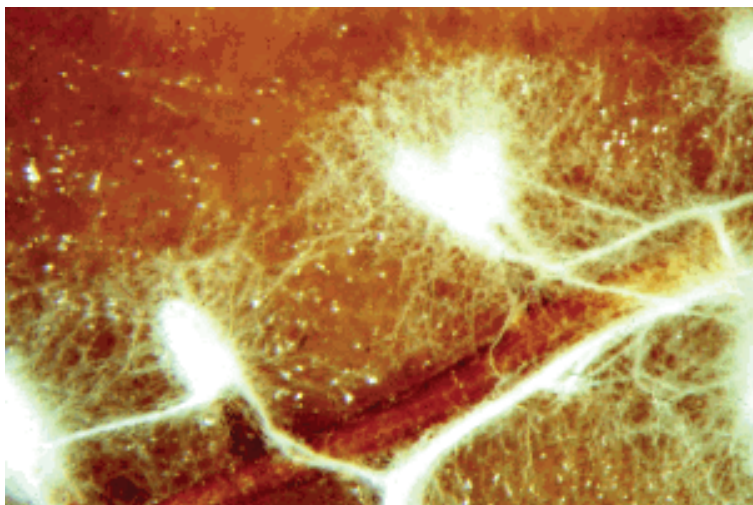


FIGURE A2-5 Ectomycorrhizal root. The hyphae of *Rhizopogon rubescens* enveloping the young roots of a Virginia pine seedling. The mycelium extends from the roots into the surrounding environment.

SOURCE: Photo by J. B. Anderson, provided by Meredith Blackwell (1996).

hemicellulose) and lignin polymers (Gilbertson, 1980). Some wood-decaying fungi invade living trees and attack non-functional tissues, especially heartwood, the non-conducting vascular tissue in the center of a cross section of the trunk. Few wood-decaying fungi actually cause diseases and most of the damage comes from the weakening of tree trunks so that they fall in wind or ice storms. The loss of weakened trees is a natural process that culls branches and entire trees to create clearings in older forests (Gilbertson, 1980). Aldo Leopold recognized the value of wood decay for wildlife in the chapter “November” of *A Sand County Almanac and Sketches Here and There*. He referred to his woodlot as “a mighty fortress that fell heir to all the diseases of plants” known to humankind. The importance of wood-decaying fungi in the formation of nesting holes for wildlife is well known (Gilbertson, 1980). The red-cockaded woodpecker prefers to nest in mature pines about 60 years old that have been rotted by the basidiomycete *Phellinus pini*. Old pine stands are a diminishing habitat in regions where pines are grown in plantations on a 15-year rotation or less for commercial use. The ivory-billed woodpecker may be extinct because the extensive old-growth, bottomland hardwood forests the species inhabited have been lost (Gilbertson, 1980).

A less significant but interesting use of wood decay is the creation of wooden objects that have been modified by wood-decaying fungi. Spalted wood is distinguished by zone lines, the dark lines formed by oxidation at the points of contact between closely related fungal colonies. The patterned wood is often favored by

collectors and increases the cost of hand-turned bowls at craft fairs. These fungal effects include the deep blue/green stain of an ascomycete fungus that remains green in intarsia of fine Italian furniture and the inlay of Tunbridge Ware objects (Blanchette et al., 1992b). Even Stradivarius violins may have been made more resonant by the partial decay of the wood (Schwarze et al., 2008).

Insects Associated with Fungi and Vertebrates

The importance of many insects in the ecosystem is overlooked, but many of them are important in degradation of coarse particles, dispersal of bacteria and fungi, and, as is well known, as agents of fungal fertilization. Fungi clearly provide benefits for insects, although the exact advantages to the fungi beyond providing habitat and a means of dispersal often are not clear (Buchner, 1965; Gilbertson, 1984; Mueller et al., 2005). Few animals have the enzymes necessary to digest refractory plant cell wall materials or to synthesize vitamins. Fungi also may detoxify plant toxins and produce pheromones for insects (Table A2-2) (Dowd, 1991; Vega and Dowd, 2005; Wheeler and Blackwell, 1984; Wilding et al., 1989). The best known fungus–insect associations include the farming interactions of basidiomycetes with Old World termites (Macrotermitinae) (Aanen

TABLE A2-2 Examples of Fungal Associations with Insects

Insect Group	Fungi	Reference
Macrotermitinae	<i>Termitomyces</i> spp.	Aanen et al. (2002)
Formicidae: Attini (derived clades)	<i>Leucocoprinus</i> spp.	Mueller et al. (2005)
Formicidae: Attini (most in <i>Apterostigma pilosum</i> clade)	Pterulaceae spp.	Munkacsi et al. (2004)
Scolytinae and Platypodinae (Bark and ambrosia beetles)	Ophiostomatoid ascomycetes	Farrell et al. (2001); Harrington (2005)
Siricidae (wood wasps)	Certain species of <i>Amylostereum</i> , <i>Stereum</i> , and <i>Daedalea</i>	Martin (1992)
Passalidae (bess beetles)	Several clades of xylose-fermenting yeasts	Suh et al. (2003)
Mushroom-feeding beetles	<i>Candida tanzawaensis</i> clade yeasts	Suh et al. (2005)
Drosophila in cacti	Various yeasts	Starmer et al. (2006)
Nectar feeding beetles	Various yeasts	Lachance et al. (2001)
Coccidae	<i>Septobasidium</i>	Henk and Vilgalys (2007)
Certain insects, especially aquatic larvae	Harpellales, Asellariales	Lichtwardt et al. (2001)

et al., 2002) and attine ants (Figure A2-6) (Formicidae: Attini) (Mueller et al., 2005) and of ascomycetes by bark and ambrosia beetles (Scolytinae and Platypodinae) (Harrington, 2005). The females of another insect group, siricid wood wasps (Siricidae), are less well studied, but they have been considered by some to form farming interactions with fungi (see Gilbertson, 1984). The interaction, however, does not meet all the criteria established for what has been defined as “agriculture” (Mueller et al., 2005).

The farming association of the basidiomycete *Termitomyces* with Old World macrotermitine termites arose once in Africa. Since that event no additional fungal lineages have been domesticated and no reversals of the fungus to a free-living state have been found. Repeated host switching, however, has occurred within termite clades as reflected in the phylogenetic trees of termites and associated fungi (Aanen et al., 2002). Nest initiation by both males and females of certain species has been suggested to have influenced the mode of transmission of the fungus, usually acquired from the environment or some source other than a parent (horizontal transmission) (Aanen et al., 2002). In the New World it is not termites, but attine ants that are involved with basidiomycetes in farming interactions, and Aanen and his colleagues (2002) compared the associations. The attines have become associated with several clades of fungi, and in contrast to termite transmission, transmission of the fungi is usually directly from parent to offspring (vertical) except in the early diverging ant lineages. Another important difference is that the ant-associated fungi apparently do not reproduce sexually. The work on the fungus–attine ant associations have revealed that ants have evolved with several groups of fungi on several different occasions. Although the best-known fungal mutualists are species of *Leucocoprinus*, other fungal groups, including certain species of Pterulaceae, have an association with ants in the *Apterostigma pilosum* clade (Munkacsı et al., 2004). The intensive studies of the fungi and attine ant associations have led to the discovery of other organisms that participate in the complex interactions. Species of hypocrealean ascomycetes in the genus *Escovopsis* are parasites of the cultivated fungus. Actinomycete associates of the ants produce antibiotics that have been reported to be specific in inhibiting *Escovopsis* (Currie et al., 1999), but more recently Sen et al. (2009) found that the bacteria they isolated had more generalized antibiotic activity, including activity against the cultivated fungus. The association of a fourth component of the association is black yeasts that apparently reduce the efficiency of the antibiotics (Little and Currie, 2008). This attine and—cultivated fungus—*Escovopsis* parasite associations provide the best example of coevolution, in this case tripartite association, among fungi and associates (Currie et al., 2003).

Unlike the termite and ant interactions, fungus-beetle associations have arisen multiple times. Some bark and ambrosia beetles have mycangia already mentioned above in which they carry inoculum of certain fungi (Malloch and Blackwell, 1993). The fungi, often *Ceratocystis* and *Ophiostoma* or relatives, may be the agents of plant diseases, and some of the fungi have been introduced



FIGURE A2-6 Excavation of deeply entrenched nest of the ant *Atta texana* requires heavy equipment or, alternatively, ground-penetrating radar to map such nests. The ant is native to adjacent parts of Texas and Louisiana, and the nests are said to be able to contain a three-story house. Visual materials based on a ground-penetrating radar nest model are available on the Internet from Carol LaFayette, Department of Visualization, Texas A&M. <http://www.viz.tamu.edu/faculty/lurleen/main/attatunnel/tunnel.html>.

SOURCE: Photo courtesy of John Moser, provided by Meredith Blackwell (2009).

with the beetles as in the case of *Raffaelea laurelensis*, the agent of laurel wilt disease (Harrington, 2005; Harrington and Fraedrich, 2010). *Ophiostoma ulmi* and similar fungi have been introduced into the United States, where they are virulent pathogens of trees, including American elms. The most efficient dispersers of some of these fungi actually were introduced before the fungus, *Ophiostoma ulmi* (Alexopoulos et al., 1996). In this discussion of beneficial fungi, these interactions benefit the insects and call attention to potential devastating effects of efficient insect dispersal in the context of emerging plant diseases.

Other beneficial fungal associates of insects involve siricid wood wasps and wood-decaying basidiomycetes, species of *Amylostereum*, *Stereum*, and *Daedalea*. The wasps lay their eggs through long ovipositors, tube-shaped organs at the posterior of the abdomen, and the larvae probably rely on fungal enzymes to decompose and detoxify the wood they ingest (Gilbertson, 1984; Martin, 1992). Many more fungi are associated with insects as necrotrophic parasites (Figure A2-7), and some of these deadly fungi have potential for development as biological control agents (Vega et al., 2009). In addition, many of about 1,000 described yeast species have close associations with insects (Table A2-2), and the yeasts provide important services to the insects (Vega and Dowd, 2005). Cer-



FIGURE A2-7 *Hirsutella citriformis* (Ophiostomataceae) on a delphacid planthopper. The asexual fruiting structure of this fungus erupted through the cuticle of the parasitized insect soon after its death.

SOURCE: Photo courtesy of Jennifer Luangsa-ard, provided by Meredith Blackwell (2010).

tain clades of gut yeasts appear to have diversified with insect hosts into certain habitats, and the yeasts provide basic resources for the insects to survive when subjected to new nutritional situations (Suh et al., 2003, 2006). About 200 species of *Septobasidium* in the Septobasidiales are known as associates of scale insects; only a few related species of *Pachnocybe* grow on wood (Henk and Vilgalys, 2007). The use of insect hosts is unusual for fungi that are related to the plant pathogenic rust fungi. The fungi are parasites of a few of the scale individuals, but in general benefit the entire insect colony by providing a protective covering against parasitic wasps (Henk and Vilgalys, 2007). Two orders of zygomycetes, Harpellales and Asellariales, were previously placed in a polyphyletic group known as Trichomycetes. The results of several studies indicate that these gut fungi produce vitamins and perhaps other benefits for their aquatic insect hosts (Lichtwardt et al., 2001). One species is known to parasitize simuliid black flies (Lichtwardt et al., 2001), potentially a benefit to those who engage in outdoor activities.

Another nutritional interaction between fungi and animals is only briefly noted here, but is extremely important. An early diverging lineage of obligately anaerobic multiflagellated fungi, the Neocallimastigomycota, and vertebrate herbivores are closely associated (Griffith et al., 2010). The fungi reside in the host rumen or another anaerobic part of the gut, where they are important in supplying cellulases and other enzymes for the degradation of the large quantities of cellulose ingested by the herbivore (James et al., 2006).

Conclusion

Many fungi are obligate, beneficial associates of other groups of organisms. These are the “good fungi” of this article, and we often fail to appreciate their value because the fungi usually are unseen within their substrates unless they form macroscopic fruiting bodies. More often it is the effects of the fungi that we observe when they ferment fruit juice, or fitting to this volume, cause dramatic new outbreaks of disease. The Robert Frost poem quoted in the prologue of this publication describes the costs of the introduction of the disease caused by the chestnut blight fungus, *Cryphonectria parasitica*. The poem predicts that the disease will ravage until a new pathogen comes to kill the fungus, and in fact a virus did appear to suppress the fungus. In 1974, however, yet another pathogen, the oriental chestnut gall wasp, was introduced to attack the trees, an additional turn not predicted by the verse.

Today, as one out of every six or seven humans on Earth is reported to be malnourished or hungry (FAO, 2010), the war against pathogenic diseases of plants and animals is as important as ever. An earlier writer, Jonathan Swift (1667–1745) addressed the topic of hunger with his essay, *A Modest Proposal*, written to bring attention to the starvation of Irish tenant farmers during the potato famine. In *Gulliver’s Travels* he wrote directly of the importance of increasing agriculture yields:

And he gave it for his opinion, “that whoever could make two ears of corn, or two blades of grass, to grow upon a spot of ground where only one grew before, would deserve better of mankind, and do more essential service to his country, than the whole race of politicians put together.”

—Jonathan Swift, *Gulliver’s Travels*, Part II, *Voyage to Brobdingnag*,
first published in 1726–1727.

This volume, *Fungal Diseases: An Emerging Threat to Human, Animal, and Plant Health*, provides a discussion of new fungal diseases of plants and the animals that we strive to overcome at a time when introduced diseases contribute to hunger.

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A3

THE FUNGI: 1, 2, 3 ... 5.1 MILLION SPECIES?^{11,12,13}

Meredith **Blackwell**^{14,15}

Premise of the Study

Fungi are major decomposers in certain ecosystems and essential associates of many organisms. They provide enzymes and drugs and serve as experimental organisms. In 1991, a landmark paper estimated that there are 1.5 million fungi on the Earth. Because only 70000 fungi had been described at that time, the estimate has been the impetus to search for previously unknown fungi. Fungal habitats include soil, water, and organisms that may harbor large numbers of understudied fungi, estimated to outnumber plants by at least 6 to 1. More recent estimates based on high-throughput sequencing methods suggest that as many as 5.1 million fungal species exist.

Methods

Technological advances make it possible to apply molecular methods to develop a stable classification and to discover and identify fungal taxa.

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Key Results

Molecular methods have dramatically increased our knowledge of Fungi in less than 20 years, revealing a monophyletic kingdom and increased diversity among early-diverging lineages. Mycologists are making significant advances in species discovery, but many fungi remain to be discovered.

Conclusions

Fungi are essential to the survival of many groups of organisms with which they form associations. They also attract attention as predators of invertebrate animals, pathogens of potatoes and rice and humans and bats, killers of frogs and crayfish, producers of secondary metabolites to lower cholesterol, and subjects of prize winning research. Molecular tools in use and under development can be used to discover the world's unknown fungi in less than 1000 years predicted at current new species acquisition rates.

What are Fungi?

Fungal biologists debated for more than 200 years about which organisms should be counted as Fungi. In less than 5 years, DNA sequencing provided a multitude of new characters for analysis and identified about 10 phyla as members of the monophyletic kingdom Fungi (Fig. A3-1). Mycologists benefited from early developments applied directly to fungi. The “universal primers,” so popular in the early 1990s for the polymerase chain reaction (PCR), actually were designed for fungi (Innis et al., 1990; White et al., 1990). Use of the PCR was a monumental advance for those who studied minute, often unculturable, organisms. Problems of too few morphological characters (e.g., yeasts), noncorresponding characters among taxa (e.g., asexual and sexual states), and convergent morphologies (e.g., long-necked perithecia producing sticky ascospores selected for insect dispersal) were suddenly overcome. Rather than producing totally new hypotheses of relationships, however, it is interesting to note that many of the new findings supported previous, competing hypotheses that had been based on morphological evidence (Alexopoulos et al., 1996; Stajich et al., 2009). Sequences and phylogenetic analyses were used not only to hypothesize relationships, but also to identify taxa rapidly (Kurtzman and Robnett, 1998; Brock et al., 2009; Begerow et al., 2010).

Most fungi lack flagella and have filamentous bodies with distinctive cell wall carbohydrates and haploid thalli as a result of zygotic meiosis. They interact with all major groups of organisms. By their descent from an ancestor shared with animals about a billion years ago plus or minus 500 million years (Berbee and Taylor, 2010), the Fungi constitute a major eukaryotic lineage equal in numbers to animals and exceeding plants (Figs. A3-2–10). The group includes molds, yeasts, mushrooms, polypores, plant parasitic rusts and smuts, and *Penicillium chrysog-*

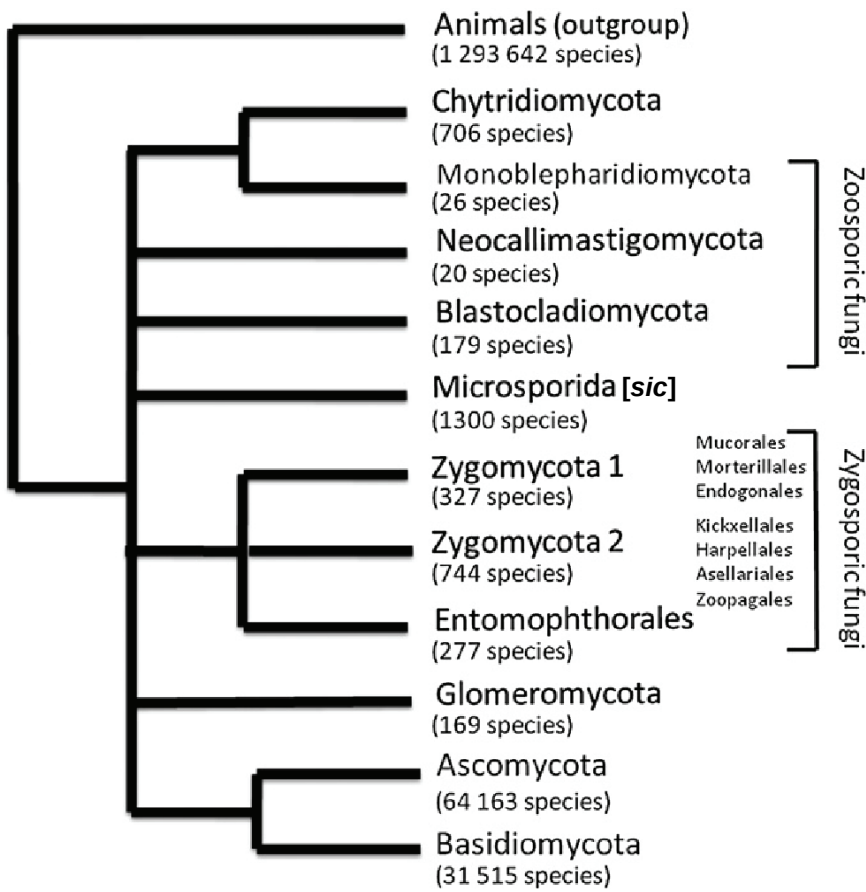


FIGURE A3-1 Fungal phyla and approximate number of species in each group (Kirk et al., 2008). Evidence from gene order conversion and multilocus sequencing indicates that microsporidians are Fungi (see below; Lee et al., 2010). Note also that zoosporic and zygosporic fungal groups are not supported as monophyletic. Tree based on Hibbett et al. (2007), White et al. (2006), and James et al. (2006).

num, *Neurospora crassa*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe*, the important model organisms studied by Nobel laureates.

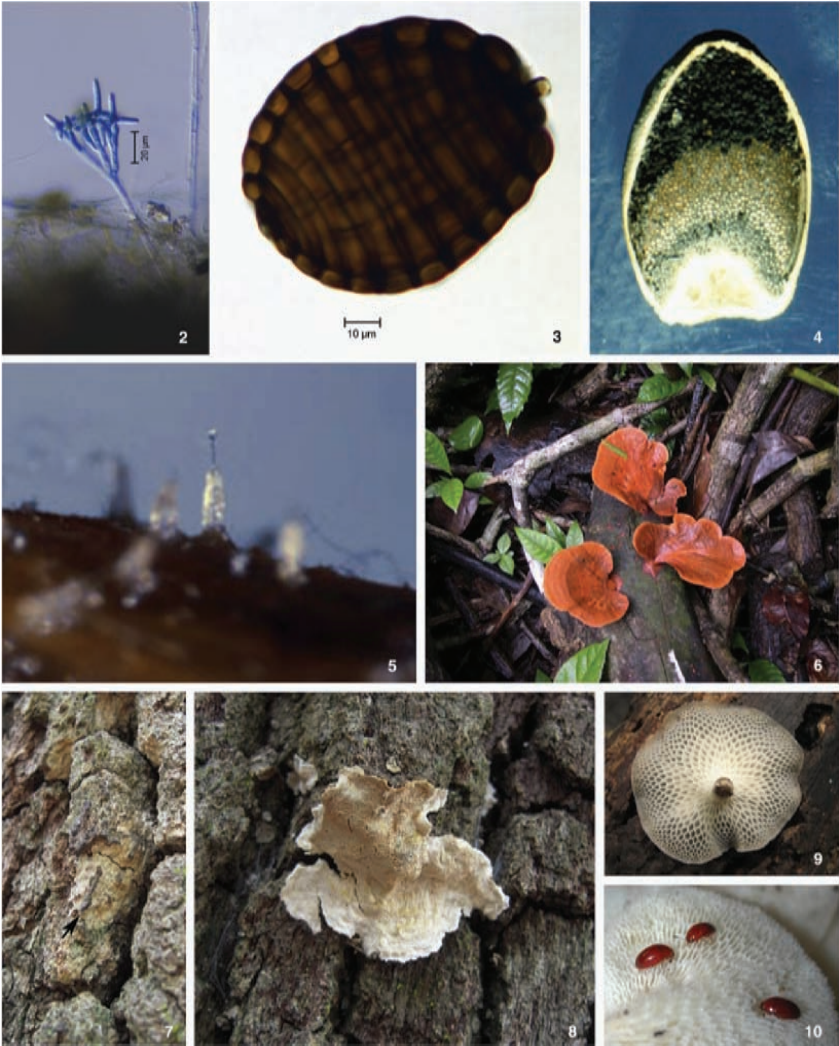
Phylogenetic studies provided evidence that nucleiid protists are the sister group of Fungi (Medina et al., 2003), nonphotosynthetic heterokont flagellates are placed among brown algae and other stramenopiles, and slime mold groups are excluded from Fungi (Alexopoulos et al., 1996). Current phylogenetic evidence suggests that the flagellum may have been lost several times among the early-diverging fungi and that there is more diversity among early diverging

zoosporic and zygosporic lineages than previously realized (Bowman et al., 1992; Blackwell et al., 2006; Hibbett et al., 2007; Stajich et al., 2009).

Sequences of one or several genes are no longer evidence enough in phylogenetic research. A much-cited example of the kind of problem that may occur when single genes with different rates of change are used in analyses involves Microsporidia. These organisms were misinterpreted as early-diverging eukaryotes in the tree of life based on their apparent reduced morphology (Cavalier-Smith, 1983). Subsequently, phylogenetic analyses using small subunit ribosomal RNA genes wrongly supported a microsporidian divergence before the origin of mitochondria in eukaryotic organisms (Vossbrinck et al., 1987). More recent morphological and physiological studies have not upheld this placement, and analyses of additional sequences, including those of protein-coding genes, support the view that these obligate intracellular parasites of insect and vertebrate hosts are members of the Fungi (Keeling, 2009; Corradi and Keeling, 2009). Additional evidence from genome structure as well as phylogenetic analyses, supports the inclusion of microsporidians within the Fungi and indicates that comparison of whole genomes contributes to the solution of challenging phylogenetic problems (Lee et al., 2010).

The level of resolution and sophistication of systematics studies made possible by molecular markers and phylogenetic analyses put mycologists on equal footing with other biologists for competitive funding, and they joined in several community-wide efforts to organize fungal diversity within a phylogenetic classification. Three projects funded by the National Science Foundation were initiated, including the Research Coordination Network: A Phylogeny for Kingdom Fungi (Deep Hypha) and successive Tree of Life projects, Assembling the Fungal Tree of Life (AFTOL-1) and a second ongoing project (AFTOL-2) (Blackwell et al., 2006). A major product of the Deep Hypha project was the publication of 24 papers on fungal phylogeny in a single journal issue (*Mycologia* 98: 829–1103). The papers included an introduction to progress in fungal phylogeny, a paper on dating the origin of Fungi, one on the evolution of morphological traits, and 21 articles with multilocus phylogenies of most major groups. Participants included 156 authors with some involved in more than one paper; only 72 of the authors were originally from North America. The multi-investigator AFTOL-1 publication (Hibbett et al., 2007) included a widely used and often cited phylogenetic classification to the level of order (e.g., Kirk et al., 2008; The NCBI Entrez Taxonomy Home-page, <http://www.ncbi.nlm.nih.gov/taxonomy>; Science Watch, <http://sciencewatch.com/dr/nhp/2009/09jannhp/09jannhpHibb>). The paper included 68 authors from more than 20 countries.

It is important to note that there was broad participation and, essentially, global involvement on these projects, emphasizing that studies of biodiversity are indeed global endeavors. Additional pages were contributed to the Tree of Life web project (<http://www.tolweb.org/Fungi/2377>) to make information on fungi more accessible to students and the general public. Two objectives of the ongoing AFTOL-2 project include increased taxon sampling of fungi for molecular



FIGURES A3-2-10 Examples of fungal diversity. **2.** *Lemonniera* sp. Tetraradiate conidia developed on a submerged leaf in a well-aerated freshwater stream surrounded by lush vegetation. This type of aquatic species, an Ingoldian ascomycete, is named for C. T. Ingold, who pioneered the study of these fungi, that are characterized by highly branched conidia. Photo courtesy of H. Raja. **3.** The aero-aquatic ascomycete *Helicoon gigantisporum* produces distinctive tightly coiled conidia. As the spore develops air is trapped in the coil and causes it to be buoyant. This feature is an adaptation for the polyphyletic aero-aquatic fungi that grow on leaves in slow-moving or stagnant freshwater. Photo courtesy of H. Raja. **4.** The smut *Testicularia* sp. develops in the ovary of grasses and (as shown here) sedges. The spores mature sequentially, with the dark spores being more mature. A plant taxonomy student once thought he had discovered a new species of *Leersia*, distinguished by large ovaries of ca. 1 cm, only to be disappointed that the enlargement was caused by a fungus. It is helpful to mycologists when plant taxonomists collect and accession fungal diversity by selecting some diseased plant specimens, an activity that should be encouraged. **5.** Perithecia of *Pyxidiophora* sp. (Laboulbeniomyces) developed in moist chamber on moose dung from Meredith Station, New Brunswick, Canada. The 150 μ m long ascospores are seen at the tip of the perithecium neck in the center. Spores adhere to phoretic mites that are carried by dung beetles to fresh dung piles. Some fungi have complex animal dispersal systems. *Pyxidiophora* species are usually mycoparasites that grow on fungi in dung or other substrates including wrack washed up on beaches. The genus is a “missing link” and provided clues to confirm that Laboulbeniomyces are ascomycetes and not other kinds of fungi or floridian red algae. **6.** The ca. 8 cm wide basidiomata of *Pycnoporus* sp., a wide-ranging, brightly colored, wood-decaying polypore, photographed at Barro Colorado Island, Panama. Some collectors have referred to basidiomycetes that produce colorful basidiomata as charismatic megamycota of the fungus world. **7.** *Peniophorella baculorubensis*, a bark-decaying basidiomycete common on and restricted to living live oak (*Quercus virginiana*), decays the bark and changes its water-holding capacity. The effect of decay on bryophyte communities by this fungus was first studied by ecologists (Penfound and Mackaness, 1940) more than 70 yr ago but was not described until a specialist on wood-decaying fungi happened to notice it on the Louisiana State University campus, Baton Rouge (Gilbertson and Blackwell, 1984). The inconspicuous basidiomata are shown growing on the lower side of a 7 cm long bark segment aimed downward for basidiospore discharge in response to gravity. **8.** Basidiomata of *Perenniporia phloiophila* on the bark of living *Quercus virginiana*. Although the basidiomata are obvious against the darker bark, this species was not described until it was discovered at the same time and often on the same trees as *Peniophorella baculorubensis*. Although the fungus usually rots only the outer bark, it will invade and decay wood whenever the vascular cambium is broached by a bird or insect. In addition to the two species on live oak, six other species have been described from the campus, illustrating the need for specialists to study noncharismatic fungi. **9.** A basidioma (8 cm diameter) of the wood-decaying fungus, *Favolus tenuiculus*, a favorite food of several species of mushroom-feeding beetles (see Fig. A3-10). Photo courtesy of N. H. Nguyen. **10.** The small (>10 mm long) brightly colored beetle, *Mycotretus* sp. (Erotilyidae), was collected at Barro Colorado Island, Panama. Many erotyloid beetles have specialized yeast-packed pouches at the anterior end of the midgut. More than 200 novel yeasts have been isolated from the gut of ca. 15 families of mushroom-feeding beetles (Suh et al., 2005). Photo courtesy of James A. Robertson.

data and the discovery of correlated morphological and biochemical characters (AFTOL Structural and Biochemical Database, <https://aftol.umn.edu>; Celio et al., 2006).

Known Fungal Species

The *Dictionary of Fungi* (Kirk et al., 2008) reported 97330 species of described fungi at the “numbers of fungi” entry. The addition of 1300 microsporidians brings the total of all described fungi to about 99000 species (Fig. A3-1). The *Dictionary*’s estimate of known species has almost tripled in the period between the first edition in 1943 (38000 described species) and now, amounting to an increase of more than 60000 described species over the 65-yr period (Fig. A3-11). Factors such as difficulty of isolation and failure to apply molecular methods may contribute to lower numbers of species in certain groups, but there cannot be any doubt that ascomycetes and basidiomycetes comprise the vast majority of fungal diversity (Fig. A3-1).

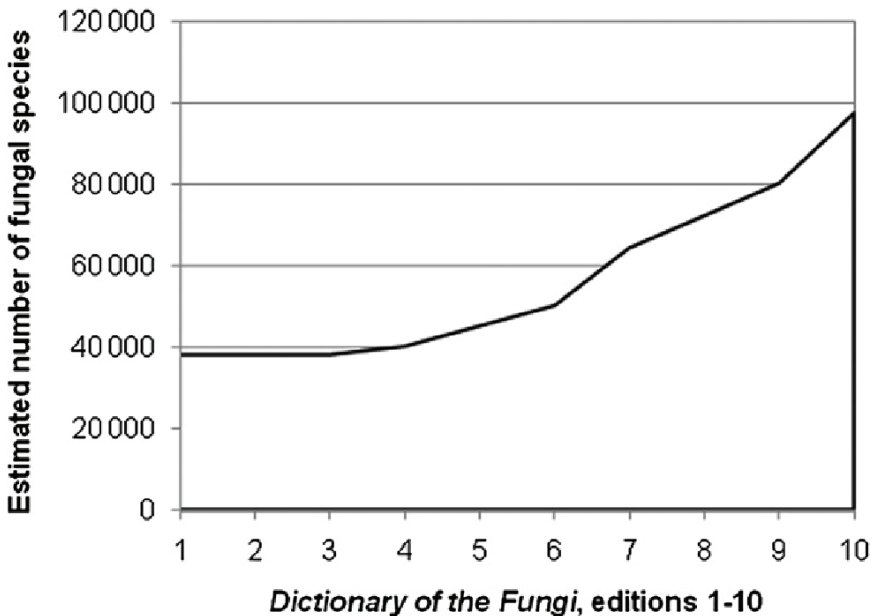


FIGURE A3-11 Numbers of known fungi from the *Dictionary of the Fungi* (editions 1–10, 1950–2008). Authors state that the large increase in species numbers in the 10th edition may be inflated because asexual and sexual forms were counted separately and molecular techniques that distinguish close taxa have been used.

Estimated total fungal numbers

In 1991, a landmark paper provided several qualified estimates of the number of fungi on the Earth based on ratios of known fungi to plant species in regions where fungi were considered to be well-studied (Hawksworth, 1991). “Estimate G” of 1.5 million species was accepted as a reasonable working hypothesis based on a fungus to plant ratio of 6:1, in contrast to the much lower 50–60-yr-old estimates by Bisby and Ainsworth (1943) of 100000 fungal species and by Martin (1951) of 250000 species based on one fungus for every phanerogam known at the time. A more recent estimate of the total number of fungi, 720 256 (Schmit and Mueller, 2007), is also low compared to present estimates that include environmental samples.

Hawksworth’s (1991) estimate now is considered to be conservative by many, including Hawksworth (Hawksworth and Rossman, 1997), because numerous potential fungal habitats and localities remain understudied (Hawksworth, 2001). Furthermore, the use of molecular methods had not yet been considered as a means of species discovery. For example, analysis of environmental DNA samples from a soil community revealed a high rate of new species accumulation at the site, and these data supported an estimate of 3.5 to 5.1 million species (O’Brien et al., 2005). Using the present discovery rate of about 1200 fungal species per year based on the last 10 years, Hibbett and his colleagues (in press) estimated that it would take 1170 years to describe 1.4 million fungi (based on Estimate G of Hawksworth [1991]) and 2840 to 4170 yr to describe 3.5 to 5.1 million (based on O’Brien et al., 2005).

Using present higher estimates of land plant numbers as somewhat under 400000 species (Paton et al., 2008; Joppa et al., 2010) fungal species numbers now are expected to outnumber land plants by as much as 10.6:1 based on O’Brien et al. (2005). Even higher ratios have been predicted using data from highthroughput sequencing of clone libraries, although individual ecosystems will vary (L. Taylor, University of Alaska, Fairbanks, personal communication, January 2011). The large gap between known and estimated species numbers has led to a series of papers and symposia (e.g., Hawksworth and Rossman, 1997; Hawksworth, 2001; Hyde, 2001; Mueller and Schmit, 2007) attempting to answer the question “Where are the missing fungi?”

How to Discover New Fungi

Collecting and culturing fungi from the environment will remain important because of the need to identify specimens, revise taxonomy, assess the roles in the environment, and provide strains for biological control, environmental remediation, and industrial processes. A physical specimen, including an inert culture, is still required as a type specimen (but see Conclusions later), and vouchers of known fungi are used for documenting DNA sequences deposited in some databases (Nilsson et al., 2006). For example, the current AFTOL project has

a requirement that each sequence deposited as part of the project be linked to a specimen, including a culture.

All taxa biological inventories (ATBIs) attempt to survey organisms within particular geographical regions by collection of specimens and culture of substrates. One of these, Discover Life in America, All Taxa Biological Inventory, seeks to survey an estimated 50000 to 100000 species of organisms in the Great Smoky Mountains National Park. Karen Hughes and Ronald Petersen have been successful in collecting more than 3000 species of fungi, mostly agarics housed in the University of Tennessee Fungal Herbarium (<http://tenn.bio.utk.edu/fungus/database/fungus-browse-results.asp?GSMNP=GSMNP>), out of about 17000 species of all taxa that have been collected by others in the park (Biodiversity Surveys and Inventories: Agaric Diversity in the Great Smoky Mountains National Park, NSF DEB 0338699). All fungal specimens have been identified, and the agarics have been studied to the extent that a culture, ITS barcode sequence, and genetic analysis are available for many species. This successful project has required hours of time over a number of years and costly resources for studying the material, but it serves as an example of the commitment needed to acquire specimen-based information on fungi.

DNA methodology makes it possible to use independent sampling methods to discover the presence of organisms without ever seeing a culture or a specimen. Several new methods significantly outperform previous automated sequencing methods (e.g., Jumpponen and Jones, 2009; Metzker, 2010). Although there may be certain limitations and biases for the different methods (Amend et al., 2010a; Tedersoo et al., 2010), mycologists have been quick to embrace them in ecological and biodiversity studies. O'Brien and colleagues (2005) pointed out that collection and culture methods revealed numbers of fungi similar to those acquired by sampling environmental DNA. Hibbett et al. (in press), however, used data from GenBank to show that by 2008 and 2009 the number of environmental samples, excluding overwhelming numbers of sequences discovered by pyrosequencing, exceeded the accessions of specimen-based sequences. The rapid development of automated, high-throughput methods also has made it possible to acquire whole genome sequences for population level studies (Liti et al., 2009; Neafsey et al., 2010).

Which Regions of the Earth Harbor Fungal Diversity?

Fungi grow in almost all habitats on Earth, surpassed only by bacteria in their ability to withstand extremes in temperature, water activity, and carbon source (Raspor and Zupan, 2006). Tropical regions of the world are considered to have the highest diversity for most groups of organisms (Pianka, 1966; Hillebrand, 2004), and this is generally true for fungi as well (Arnold and Lutzoni, 2007).

A group of researchers are studying the diversity of the Guyana Shield. For the last 11 years, Terry Henkel and Cathie Aime and their colleagues have studied the fungi in six 1-km² plots—three in a *Dicymbe corymbosa*-dominated

forest and three in a mixed tropical forest. Their current collections contain 1200 morphospecies, primarily basidiomycetes. Approximately 260 species were collected repeatedly only in the *Dicymbe* plots. Thus far, two new genera and ca. 50 new species have been described. On the basis of groups already studied, Aime estimated that ca. 120 new ectomycorrhizal taxa have been discovered. Including novel saprobes as well as ectomycorrhizal fungi, ca. 500 new species are expected among the 1200 taxa collected. It is clear, however, that these are not simply high numbers of new taxa, but biologically interesting fungi as well (Aime et al., 2010). One species is so unusual, that a reviewer of the original report called it “the find of the century” (Redhead, 2002). As Aime has quipped “if one were to compare the ratio of fungi to plants in the *Dicymbe* plots as did Hawksworth (1991), the ratio would be 260 to 1, obviously an overestimate but also a cautionary exercise in basing any estimate on a single ecotype” (M. C. Aime, Louisiana State University, personal communication, August 2010).

Many fungi have in fact come from temperate regions, and some studies report a high diversity of fungi. For example, in a study of indoor air from buildings using culture-independent sampling methods, diversity was found to be significantly higher in temperate sites independent of building design or use. The authors also alluded to the possibility that previous studies of certain mycorrhizal fungi showed similar trends (Amend et al., 2010b). More investigation in this area is needed, but it is clear that many undescribed fungi are present in temperate regions. Popular literature often rationalizes the need to save the rainforests, not because of their intrinsic value, but because of the potential drug-producing organisms that may be found there. Many of the commercially most successful fungal drugs, however, come from temperate fungi. *Penicillium chrysogenum*, producer of penicillin, was found in a northern temperate city. Another remarkable fungus, *Tolypocladium inflatum* from Norwegian soil, synthesizes cyclosporine, an immune-suppressant drug that revolutionized organ transplants (Borel, 2002); the sexual state of this fungus was collected in New York, USA (Hodge et al., 1996). Today the drug is commonly used to treat dry eye (Perry et al., 2008), as well as many serious conditions. Statins produced by fungi such as *Aspergillus terreus* from temperate regions, combat high cholesterol levels, as well as providing other benefits (Vaughan et al., 1996; Askenazi et al., 2003; Baigent et al., 2005).

In temperate deserts, mycorrhizal boletes, agarics, and rust and smut fungi, are common. A surprising number of wood-decaying basidiomycetes have been discovered on living and dead desert plants, including cacti and are in the University of Arizona, Robert L. Gilbertson Mycological Herbarium (<http://ag.arizona.edu/mycoherb/herbholdings>). When a noted mycologist moved to Arizona early in his career, he became excited about the new and unreported fungal diversity found in the desert. His proposed study of the wood-decaying fungi of the Sonoran Desert was poorly received with a comment that wood-decaying fungi were not present in the desert (R. L. Gilbertson, University of Arizona, personal communication, August 1979). The Sonoran Desert, however, has many plants

(e.g., cacti, ocotillo, and mesquite and other desert legumes) that are substrates for polypores and resupinate basidiomycetes (e.g., Gilbertson and Ryvarden, 1986, 1987).

Fungi also grow at low temperatures. An example involves fungal deterioration of historic huts built between 1901 and 1911 for use by Antarctic explorers including Robert Scott and Ernest Shackleton, and although there are not large species numbers, it is important not to overlook this fungal habitat in diversity studies (Held et al., 2005). Lichens have often been reported to be common in Arctic and Antarctic regions (Wirtz et al., 2008), and yeasts are active under frozen conditions in the Antarctic (Vishniac, 2006; Amato et al., 2009). In some cases, a yeast isolated from the Antarctic (based on 28S rDNA barcoding) also has been reported from varied habitats, including human infections, the gut of insects, deep seas, and hydro-carbon seeps (Kurtzman and Fell, 1998; Bass et al., 2007; personal observation). Although some fungi are specialized for cold regions, others simply occupy a wide variety of environmental conditions.

Many regions and habitats of the world need to be included in fungal discovery. In general, microscopic fungi and those that cannot be cultured are very poorly known. Parts of Africa remain to be collected for many, although not all, fungal groups (Crous et al., 2006). Fungi are important as symbionts, and they are associated with every major group of organisms, bacteria, plants and green algae, and animals including insects. Because certain under-studied symbiotic associations are known to include large numbers of fungi, these are a good place to search for new taxa. The associated organisms also allow for resampling, a quick way to obtain data about host specificity. Targeting hosts also is a productive method for discovering fungal fossils, such as those associated with plants of the Rhynie Chert (Taylor et al., 2004). Examples of diversity in particular fungal habitats are reviewed in the following sections.

Fungi and Plant Roots

Mycorrhizal plants and their fungal partners have been studied by a number of mycologists (Trappe, 1987; Smith and Read, 2008). The fungi often are essential to their plant hosts because they take up water, nitrogen, phosphorus, and other nutrients from the soil and transfer them to the plant roots. Some of these fungi may not prosper or even grow without the host. In addition to flowering plants and conifers, many bryophytes and ferns are mycorrhizal (Pressel et al., 2010). Certain mycorrhizal fungi specialize on orchids and ericoid plants, and some are known to have invaded new habitats with successful invasive plants (Pringle et al., 2009).

There are two main types of mycorrhizal fungi, arbuscular mycorrhizae (AM) and ectomycorrhizae. AM associations are more common and occur with up to 80% of all plant species and 92% of plant families. AM fungi are all members of the phylum Glomeromycota, a less diverse group than ectomycorrhizal

fungi with about 250 described species in a variety of taxa (Gerdemann, 1968; Schüssler and Walker, 2011; Wang and Qiu, 2006). Evidence from recent molecular studies, however, indicates that cryptic species with higher levels of host specificity than previously realized will increase the number of known AM fungi (Selosse et al., 2006; Smith and Read, 2008). More than 6000 species, mostly of mushroom-forming basidiomycetes, form ectomycorrhizae with about 10% of all plant families. Greater host specificity usually occurs in the ectomycorrhizal fungus–plant associations than in AM associations (Smith and Read, 2008). Vast parts of the world remain to be sampled (Mueller et al., 2007), and it is expected that barriers to inter-breeding have led to high genetic diversity among these fungi (Petersen and Hughes, 2007).

Inside Plant Leaves and Stems

Almost all plants on Earth are infected with endophytes, fungi that do not cause disease symptoms (Saikkonen et al., 1998). Endophytes occur between the cells, usually of above ground plant parts, and represent a broad array of taxonomic groups (Arnold, 2007; Rodriguez et al., 2009). The earliest studies of endophytes were of those associated with grasses (Diehl, 1950). Some grass endophytes are specialized members of the Clavicipitaceae, relatives of insect and fungal parasites in the Hypocreales, and many species produce alkaloid toxins effective against insects, other invertebrate animals, and vertebrates (Clay et al., 1993). Some grass endophytes are transmitted to the host offspring in seeds, and others inhibit sexual reproduction in the host and are dispersed within plant parts such as leaf fragments. For grass endophytes that reproduce sexually, fertilization may occur by insect dispersal. Water intake is increased in infected hosts, and these plants often grow taller than uninfected hosts.

A much more diverse group of endophytic fungi are associated with plants in addition to grasses, including a variety of dicots and conifers (Carroll, 1988; Rodriguez et al., 2009). In some tropical forests considered to be diversity hot-spots for endophytes, there are extremely large numbers of the fungi, sometimes with hundreds reported from a single tree species, judged by both cultural and molecular methods of discovery and identification (Arnold et al., 2001; Arnold and Lutzoni, 2007; Pinruan et al., 2007; Rodriguez et al., 2009). In one study, more than 400 unique morphotypes were isolated from 83 leaves of two species of tropical trees. A subset of the fungi was distributed among at least seven orders of ascomycetes (Arnold et al., 2000). Leaves usually acquired multiple infections as they matured, and there was strong evidence that the endophytes protected leaves of plants, such as *Theobroma cacao*, from infection when they were challenged with pathogens (Arnold et al., 2003). Vega and colleagues (2010) also found high diversity of endophytes in cultivated coffee plants. Interestingly, some of these were insect pathogens and experiments are being conducted to develop endophytes as biological control agents of insect pests.

Plant Pathogens

Plant pathogens differ from endophytes in that they cause disease symptoms. Although some zoosporic and zygosporic fungi are plant pathogens, most plant pathogens are ascomycetes and basidiomycetes. A large number of ascomycetes and ca. 8000 species of basidiomycetes are plant pathogens. In addition to crop pathogens, it is important to remember that many pathogens are numerous and important in natural ecosystems (Farr et al., 1989; Burdon, 1993). Nonpathogenic phylloplane yeasts occupy leaf surfaces of many plants and are increasingly recognized for their control of potential leaf pathogens (Fonseca and Inácio, 2006). In addition to the thousands of native fungi that parasitize plants in the United States, pathologists are constantly on the lookout for introduced pathogens that often are undescribed when they arrive to decimate naïve native plant populations. For example, invasive fungi such as those grouped as Dutch elm disease fungi, chestnut blight fungus, dogwood anthracnose fungus, and redbay wilt fungus, were all unknown until they were observed soon after their introduction (Alexopoulos et al., 1996; Zhang and Blackwell, 2001; Harrington et al., 2008). Exotic localities will need to be searched for undescribed fungi that probably go largely unnoticed on their native hosts. It is important to note that although fungi may cause only minor symptoms to hosts in their native habitats, one of these may have the potential to be the next destructive disease after introduction to a new region.

Molecular methods have helped to clarify limits of closely related species and to establish host ranges (e.g., Crous et al., 2008). In a study of 26 leaf spot fungi in Australia, three genera of Myrtaceae, including *Eucalyptus*, were hosts for three new genera and 20 new species (Cheewangkoon et al., 2009). Although the authors acknowledged the high level of new taxa discovered, they pointed out that the potential for host shifts within plantations might lower estimates of fungal species numbers worldwide. Host or substrate specificity is a concept that can be applied to fungal groups that are closely associated with hosts such as endophytes, pathogens, and mycorrhizal fungi but not usually for saprobic species (Zhou and Hyde, 2001). In the past species of plant pathogens often were based on host identity, a practice that is not always effective because some groups are host-specific while others are not.

Lichens and Lichenicolous Fungi

About 20% of all fungi and 40% of the ascomycetes (13500 species) are lichen-forming fungi (Lutzoni and Miadlikowska, 2009). Lichenicolous fungi, parasites, and other associates of lichens are not well collected, but an estimate for the combined lichens and lichenicolous fungi is about 20000 species (Feuerer and Hawksworth, 2007). Lichens and lichenicolous fungi are polyphyletic, and several different groups of ascomycetes and a few species of basidiomycetes have become associated with green algae and cyanobacteria (Lutzoni and

Miadlikowska, 2009). Feuerer (2010) can be consulted for information on lichen diversity worldwide. This checklist also highlights the absence of collections in certain regions.

Deserts are rich in lichens. Of 1971 lichen species and associated fungi reported from the Sonoran Desert, about 25% studied since 1990 are new. Three volumes on lichens of the greater Sonoran Desert region have been published (Nash et al., 2002, 2004). Other habitats of high lichen diversity are Arctic and Antarctic regions (Feuerer, 2010).

Fungi From Arthropod and Invertebrate Animals

There is a need for more information on arthropod- and insect-associated fungi. As was mentioned earlier, estimates of global fungal diversity usually omit insect-associated species because they are so poorly known (Hawksworth, 1991; Rossman, 1994; Mueller and Schmit, 2007; Schmit and Mueller, 2007). Several post-1991 estimates of insect-associated fungi suggested that 20 000–50 000 species exist (Rossman, 1994; Weir and Hammond 1997a, b; Schmit and Mueller, 2007). Some parasites are biotrophic, associated with living insects, and many do not grow in culture. These also usually require special methods for removal and mounting, and few mycologists or entomologists have ever seen members of the Laboulbeniomyces or the fungal trichomycetes, Asellariales and Harpellales (Lichtwardt et al., 2001; Cafaro, 2005). Laboulbeniomyces are seta-sized, ectoparasitic ascomycetes of insects, mites, and millipedes (Weir and Blackwell, 2005). All 2000 known species have distinctive life cycles with determinate thalli arising from two-celled ascospores. About 90% of the species have been found on adult beetles (12 of 24 superfamilies) or on flies. New arthropod hosts at the level of family are still being discovered (Weir and Hammond, 1997a, b; Rossi and Weir, 2007), and there is an indication that there is some degree of host specificity (De Kesel, 1996). In the future, increased use of molecular methods will make it possible to determine the degree of species level host specificity, but the information is not available now. Septobasidiales, relatives of the basidiomycete rust fungi are associated with scale insects, and their felty basidiomata presumably protect the insects from parasitoid wasps. Many microsporidians also are parasites of a broad group of host insects.

Necrotrophic parasites of insects include some members of Chytridiomycota, Blastocladales (*Coelomomyces*), Entomophthorales, and Tubeufiaceae (*Podonectria*) (Benjamin et al., 2004). About 5000 members of three families of Hypocreales are necrotrophic parasites of arthropods (Spatafora et al., 2007, 2010). These species show an evolutionary pattern of host shifting among plants, fungi, and insects in addition to displaying a high level of host specificity.

Fungi also occur in ancient, obligate gardening associations with bark and ambrosia beetles, attine ants, and Old World termites, and new species are still being discovered in these groups (Benjamin et al., 2004; Little and Currie, 2007;

Harrington et al., 2008; Aanen et al., 2009). Many yeasts are associated with insects, particularly insects that feed on nectar (Lachance, 2006; Robert et al., 2006).

Other insects contain gut yeasts, a habitat where few have looked for them. Isolations from the gut of mushroom-feeding beetles yielded up to 200 new species of yeasts (Suh et al., 2004, 2005; see also Lachance et al., 2010). Because only about 1500 ascomycete yeasts (Saccharomycotina) have been described, the gut yeasts represent a dramatic increase in diversity from a limited geographical range (Boekhout, 2005; C. Kurtzman, USDA-ARS, personal communication, July 2010). In fact, the estimated total number of yeast species worldwide could be increased by as much as 50% by simply recollecting in previously collected sites from the study (Suh et al., 2005). As Lachance (2006) pointed out, based on predictions of yeast numbers using data from species in slime fluxes and in associations with flower-visiting insects, it is necessary to obtain more information on specificity and geographical ranges before better estimates can be made. Although not all insects harbor large numbers of yeasts in their guts, those with restricted diets in all life history stages such as mushrooms or wood are often associated with yeasts. Host insects may acquire digestive enzymes or vitamins from the yeasts. This contention is supported by the fact that unrelated insects feeding on mushrooms (e.g., beetles in different lineages, lepidopteran larvae) all have gut yeasts with similar assimilative capabilities and vitamin production. The high rate of discovery of yeasts in under-collected habitats and localities suggests that far more taxa await discovery (Suh et al., 2005), and the gut habitat has been considered a yeast diversity hotspot (Boekhout, 2005).

Insects may be food for fungi, especially in low nitrogen environments. The mycelium of *Pleurotus ostreatus*, a favorite edible species for humans, secretes toxic droplets that kill nematodes. A study involving the mushroom-producing, ectomycorrhizal basidiomycete, *Laccaria bicolor*, was designed to determine the amount of predation by springtails on the fungal mycelium. The study led to the surprise discovery that the fungus was not insect food, but rather, it, and indirectly, the host tree benefited by obtaining substantial amounts of nitrogen from the insects (Klironomos and Hart, 2001). The predatory habit has arisen independently on several occasions in at least four phyla of fungi and oomycetes. Predaceous fungi such as species of *Arthrobotrys* and *Dactylella* lure, then trap, snare, or grip nematodes and other small invertebrate animals in soils and in wood (Barron, 1977).

Ødegaard (2000) revised global estimates of arthropods downward from 30 million to 5–10 million. Not all insects and arthropods are tightly associated with fungi, but even the revised species estimates indicate that the numbers of insect-associated fungi will be very high.

Soil Fungi

Soil is a habitat of high fungal diversity (Waksman, 1922; Gilman, 1957; Kirk et al., 2004; Domsch et al., 2007). Soil fungi and bacteria are important in biogeochemical cycles (Vandenkoornhuyse et al., 2002), and the diversity of soil fungi is highest near organic material such as roots and root exudates. Per volume, large numbers of microscopic fungi occur in pure soil, and these are largely asexual ascomycetes and some zygomycetes, including animal-associated Zoopagales. Gams (2006) estimated that 3150 species of soil fungi are known, and ca. 70% are available in culture. There presently is a high rate of new species acquisition, and the group appears to be better known than most ecologically defined groups. Molecular studies, however, are predicted to increase the total number (Bills et al., 2004). In fact a study of soil communities in several forest types at the Bonanza Creek Long Term Ecological Research site, Fairbanks, Alaska, United States, revealed not only seasonal changes in community composition but also in dominance of fungi over bacteria. The data acquired by several molecular methods including high-throughput sequencing greatly increased the total number of fungal sequences in GenBank at the time (Taylor et al., 2010). Taylor and his colleagues found more than 200 operational taxonomic units in a 0.25 g soil sample with only 14% overlap in a sample taken a meter away. This study is not directly comparable with the soil fungi reported by Gams (2006) because Gams' figures excluded fungi such as mycorrhizal species.

Another study of soil fungi based on environmental DNA sequences showed an unexpected distribution of a group of zoosporic fungi, Chytridiomycota. The chytrids, were found to be the predominate group of fungi in nonvegetated, high-elevation soils at sites in Nepal and in the United States in Colorado, where more than 60% of the clone libraries obtained were from chytrids. A phylogenetic analysis of the sequences compared with those of a broad selection of known chytrids, indicated that a diverse group of Chytridiomycota representing three orders was present (Freeman et al., 2009).

Most major fungal lineages are known from cultures and specimens, but there have been a few surprises even in well-sampled habitats such as soil. Soil clone group I (SCGI) represents a major lineage of fungi that occurs in temperate and tropical soils on three continents, but no one has ever seen or isolated any of the species into culture (Schadt et al., 2003; Porter et al., 2008).

The phylogenetic position of this lineage, perhaps a new phylum, appeared as a sister group to the clade of Pezizomycotina–Saccharomycotina (Porter et al., 2008). Other unexpected higher taxonomic level fungal clades have been detected from environmental DNA sequences (Vandenkoornhuyse et al., 2002; Jumpponen and Johnson, 2005; Porter et al., 2008). Another lineage detected by environmental sequences was subjected to fluorescent in situ hybridization (FISH). The outline of a single-celled, flagellated organism was detected (Jones and Richards, 2009), but apparently none of these fungi has been cultured either. Higher-level

bacterial taxa have been discovered by environmental sampling, but this is a far less common occurrence for fungi (Porter et al., 2008).

Fungi form crusts that stabilize desert soils. Crusts usually are made up of darkly pigmented ascomycetes, lichens, and nitrogen-fixing cyanobacteria (States and Christensen, 2001). Rock-inhabiting fungi occur in the surface and subsurface layers of desert rocks. These darkly pigmented ascomycetes are members of the classes Dothideomycetes and Arthoniomycetes, but basidiomycetes and bacteria may occur in the associations (Kuhlman et al., 2006; Ruibal et al., 2009). Easily cultured asexual ascomycetes and other fungi also occur in desert soils, and these include an unusual zygomycete, *Lobosporangium transversale* (Ranzoni, 1968), known only from three isolations including Sonoran Desert soil. Yeasts are well known from American deserts in association with cacti and flies where they detoxify plant metabolites (Starmer et al., 2006).

Freshwater Fungi

Certain fungi are adapted for life in fresh water. More than 3000 species of ascomycetes are specialized for a saprobic life style in freshwater habitats where they have enhanced growth and sporulation (Shearer et al., 2007; Kirk et al., 2008; Shearer and Raja, 2010). The asci are evanescent, and ascospores have appendages and sticky spore sheaths, that anchor the spores to potential substrates in the aquatic environment. Conidia have several dispersal strategies, and these are designated as Ingoldian (Fig. A3-2) and aero-aquatic (Fig. A3-3) conidia. Ingoldian conidia are sigmoidal, branched, or tetra-radiate and attach to plants and other material in the water. The conidia float on foam that accumulates at the banks of streams, especially during heavy runoff, and when the bubbles burst, the spores may be dispersed for great distances from the water and into trees, where they can be isolated from water-filled tree holes (Bandoni, 1981; Descals and Moralejo, 2001; Gönczöl and Révay, 2003). Aero-aquatic fungi have multicellular, often tightly helical conidia with air spaces to make them buoyant on the surface of slower-moving waters (Fisher, 1977).

Other, less obviously modified fungi are present in water, and some of these are active in degrading leaves in streams after the heavy autumn leaf fall. A few specialized freshwater basidiomycetes also are known, and several have branched conidia similar to those of the Ingoldian ascomycetes. Flagellated fungi occur in aquatic habitats, including Chytridiomycota, Blastocladiomycota, and Monoblepharomycota (James et al., 2006). *Batrachochytrium dendrobatidis*, the recently described amphibian killer, is an aquatic chytrid (Longcore et al., 1999). Members of Neocallimastigomycota also live in a specialized largely aquatic environment, the gut of vertebrate herbivores, where they are essential for digestion of cellulosic substrates.

Marine Fungi

Marine waters provide a habitat for certain specialized fungi (Kohlmeyer and Volkmann-Kohlmeyer, 1991), and Hyde et al. (1998) estimated that more than 1500 species of marine fungi occur in a broad array of taxonomic groups. Many of these fungi are distinct from freshwater aquatic species, and they may be saprobic on aquatic plant substrates. Some species have characters such as sticky spore appendages, indicators of specialization for the marine habitat (Kohlmeyer et al., 2000).

It is interesting that few fungi from early-diverging lineages have been reported from marine environments, perhaps in part because mycologists studying these groups sampled more often from fresh water habitats. More recently, an investigation of deep-sea hydrothermal ecosystems revealed not only novel species of ascomycetes and basidiomycetes, but also what may be a previously unknown lineage of chytrids (Le Calvez et al., 2009).

Most marine fungi are ascomycetes and basidiomycetes, and these include ascomycete and basidiomycete yeasts (Nagahama, 2006). Some of the yeasts degrade hydrocarbon compounds present in natural underwater seeps and spills (Davies and Westlake, 1979). Certain ascomycetes are specialists on calcareous substrates including mollusk shells and cnidarian reefs. Even a few mushroom-forming basidiomycetes are restricted to marine waters (Binder et al., 2006). Some fungi use other marine invertebrates as hosts (Kim and Harvell, 2004), including antibiotic producers that live in sponges (Bhadury et al., 2006; Pivkin et al., 2006; Wang et al., 2008). A wide variety of fungi considered to be terrestrial also are found in marine environments. Basidiomycete (i.e., *Lacazia loboi*) and ascomycete yeasts, and other fungi including *Basidiobolus ranarum*, may occur in marine waters where they infect porpoises and other vertebrates (Kurtzman and Fell, 1998; Murdoch et al., 2008; Morris et al., 2010).

Fungal Species

Currently, molecular methods provide large numbers of characters for use in phylogenetic species discrimination (e.g., Kohn, 2005; Giraud et al., 2008). In the past, biologists relied primarily on phenotype for species delimitation, and most of the formally described species known today were based on morphology. In addition, mating tests have been used to distinguish so-called biological species, especially among heterothallic basidiomycetes (Anderson and Ullrich, 1979; Petersen, 1995). The ability to mate, however, may be an ancestral character. For example, Turner et al. (2010) found evidence that fungi have evolved strong barriers to mating when they have sympatric rather than allopatric distributions. Distant populations would not have had strong selective pressure against hybridization, thereby avoiding production of progeny less fit than conspecific progeny (e.g., Garbelotto et al., 2007; Stireman et al., 2010). This phenomenon, known as reinforcement, helps to explain how fungi from different continents can mate

in the laboratory but never in nature and is an argument in favor of recognizing species by phylogenetics. A number of researchers have recognized species using “phylogenetic species recognition” criteria (Taylor et al., 2000). The operational phylogenetic method is based on a “concordance of multiple gene genealogies,” and in addition to discriminating species, the method indicates whether fungal populations actually exchange genes in nature (Taylor et al., 2000; Fisher et al., 2002; Dettman et al., 2006; Jacobson et al., 2006).

The use of phylogenetic species criteria results in recognition of more species than those delimited by morphological characters. For example, work on *Neurospora* species resulted in the discovery of 15 species within five previously recognized species (Dettman et al., 2006; Villalta et al., 2009). There are many such examples among other groups of fungi, and eventually these may be a significant source of new species discovery in the effort to discover 5 million fungi. Fungal species recognized in this way may be described without a phenotypic diagnosis, but it is not uncommon for distinguishing characters to be found with guidance from the phylogenetics study (e.g., Otrosina and Garbelotto, 2010).

Conclusions

Until recently, estimates of numbers of fungi did not include results from large-scale environmental sequencing methods. Newer estimates based on data acquired from several molecular methods, however, have predicted as many as 5.1 million species of fungi (O’Brien et al., 2005; Taylor et al., 2010). Mycologists also are beginning to use high-throughput methods to gain insight into questions including geographical ranges and host and substrate specificity, topics that have direct bearing on species numbers (Lumbsch et al., 2008). For example, high-throughput methods have been used to determine the amount of overlap between species within a given region by comparing soil samples a meter apart to find only 14% species overlap (Taylor et al., 2010).

A better estimate of fungal numbers also can be speeded by enlisting more biologists to accomplish the goal. When amphibian populations first were observed to be dwindling and some species were determined to have disappeared almost 20 yr earlier, a number of causes, all nonfungal, were suggested as the explanation. The revelation that a chytrid was involved brought to mind that there were probably fewer than 10 mycologists in the world who could collect, isolate, culture, and identify the novel flagellated fungus, *Batrachochytrium dendrobatidis* (Longcore et al., 1999). Since that time interest in and publications on chytrids have increased dramatically (e.g., Freeman et al., 2009; LeCalvez et al., 2009). The interest in amphibian disease was in part the impetus for a large number of recent publications on amphibian decline, but amphibian decline also justified other projects, including training new chytrid systematists in monographic work. This effort has resulted in the discovery of many new chytrid species and the description of five new orders between 2008 and 2010. The rise of AIDS and the

accompanying large number of fungal infections brought about a similar interest in medical mycology several decades ago.

In addition to any sudden influx of biologists to obtain better estimates of fungal numbers, a new approach clearly is needed. In a thoughtful paper, Hibbett and colleagues (in press) called for obtaining clusters of similar sequences and assigning Latin binomials to these molecular operational taxonomic units (MOTUs). The names would allow the sequences to be integrated into a specimen-based taxonomic data stream. They considered inclusion of the sequence-based taxa among all taxa to be a better alternative than the candidate taxon status used by bacteriologists. Changes in the International Code of Botanical Nomenclature would be needed if sequence-based materials were to be allowed as nomenclatorial types. This proposal seems to be a practical approach to handling the overwhelming fungal diversity being discovered.

Recent experience in working as a broadly inclusive group to plan and produce a phylogenetic classification, the development of freely accessible databases, and the use of new tools to survey fungi in ecological studies has prepared the mycological community to accomplish a number of new goals, including the discovery of millions of fungi.

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A4

BAT WHITE-NOSE SYNDROME IN NORTH AMERICA¹⁶

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Since 2007, infections by a previously unrecognized, perhaps imported fungus killed an estimated 1 million bats in North America.

Summary

- **The newly described fungus, *Geomyces destructans*, causes an invasive skin infection in bats and is the likely agent of white-nose syndrome (WNS).**

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- **With immune system functions and body temperatures reduced during hibernation, bats may be unusually susceptible to a pathogenic fungus such as *G. destructans*.**
- **WNS was first observed in a popular show cave near Albany, New York, leading some investigators to suspect that a visitor inadvertently introduced *G. destructans* at this site, triggering a wider WNS outbreak in North America.**
- **Biologists trying to manage WNS within North American bat populations face major challenges, including the variety of susceptible host species, incredible dispersal capabilities of bats, difficulties in treating such populations, and persistence of the pathogen in their vulnerable underground habitats.**

In 2007 bats in eastern North America began dying in unprecedented numbers from a previously undocumented disease, now called white-nose syndrome (WNS). Although the ecological and economic impacts of this disease are not fully elucidated, this severe loss of insectivorous bats threatens decreased crop yields, forest defoliation, and a rise in insect-borne diseases. The recent emergence of WNS in bats of eastern North America, its rapid spread, and the severity of the outbreak highlight the importance of wildlife disease as an integral component of ecosystem health.

Biologists with the New York State Department of Environmental Conservation first recognized WNS as a problem in late winter 2007 at five hibernation sites near Albany, N.Y. Subsequently, a recreational caver furnished a photograph from February 2006 in nearby Howes Cave depicting bats with clinical signs of WNS, implicating this location as the likely index site and suggesting disease emergence the winter before New York state biologists drew public attention to the disease. By 2011 WNS had spread south along the Appalachian Mountains into eastern Tennessee, as far west as southern Indiana and western Kentucky, and north into the Canadian provinces of Quebec, Ontario, and New Brunswick (Figure A4-1). Experts estimate that more than 1 million bats have died from WNS thus far. Modeling studies show that, if such mortality trends continue, one of the most abundant bat species in eastern North America, the little brown bat (*Myotis lucifugus*), could disappear from this region within 16 years. Sustained killing of this magnitude from an infectious disease is unprecedented among the approximately 1,100 species of bats known worldwide.

The Host, Pathogen, and Environment

The likely agent of WNS is a newly described fungus, *Geomyces destructans*, which causes an invasive skin infection that is the hallmark of this disease (Figure A4-2). *G. destructans* belongs to the order Helotiales within the phylum Ascomycota. Characteristics that distinguish it from other *Geomyces* spp. include

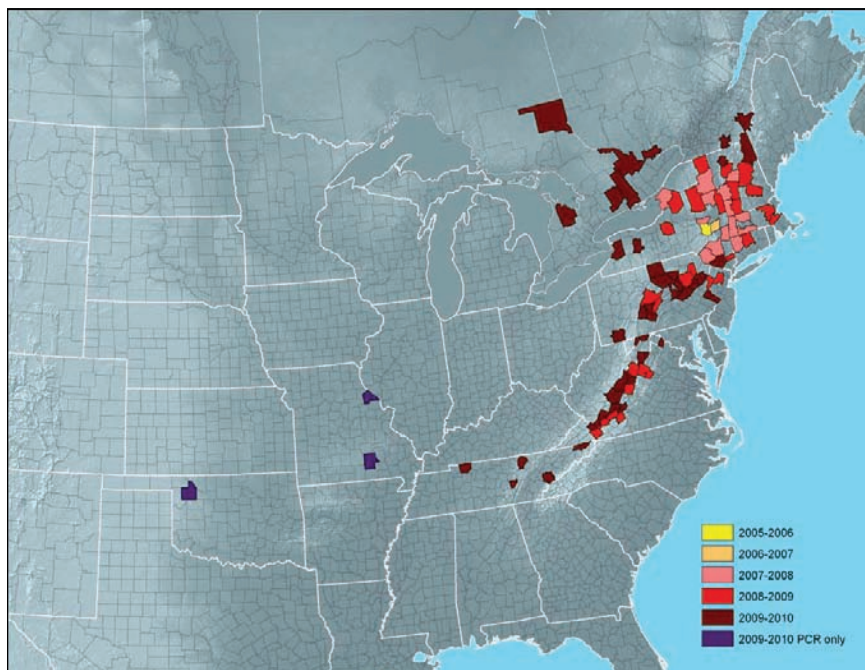


FIGURE A4-1 Occurrence of white-nose syndrome and/or *Geomyces destructans* in the United States (by county) and Canada (by county or district) from winter 2005–2006 through April 2011.

curved conidia (Figure A4-2), slow growth on laboratory medium, cold adaptation, and pathogenicity to bats. Species of *Geomyces* exist in soils worldwide, especially in colder regions.

Any infectious disease involves interactions among a susceptible host, pathogen, and the environment. To comprehend the ecology of WNS, we must consider the physiological and behavioral aspects of bats that make them susceptible to the disease, the characteristics of the fungus that allow it to act as a pathogen, and the role of underground sites (hibernacula) such as caves and mines in providing conditions conducive to maintaining this pathogen and enabling it to infect these hosts.

WNS appears to occur only in bats, suggesting they possess unique traits that make them a suitable host. Bats are nocturnal and the only mammals capable of powered flight. Their forelimbs are highly modified, consisting of elongated phalanges connected by a thin layer of skin to form wings. This body plan provides bats with selective advantages that allow them to dominate the night skies, making them the second most diverse group of mammals, accounting for approximately 1,100 of 5,400 mammalian species. Of 45 bat species in the United



FIGURE A4-2 Micrograph of *Geomyces destructans* showing distinctive asymmetrically curved conidia either free or borne singly at the tips and sides of branched conidiophores (bar, 10 μm).

States, at least 6 of the approximately 25 that hibernate have been documented with WNS, including the little brown bat, the northern long-eared bat (*M. septentrionalis*), the eastern small-footed bat (*M. leibii*), the endangered Indiana bat (*M. sodalis*), the tricolored bat (*Perimyotis subflavus*), and the big brown bat (*Eptesicus fuscus*). All six of those species are insectivorous and cope with winter food shortages by hibernating in cold and humid, thermally stable caves and mines. When hibernating, the animals typically congregate in large numbers, dramatically reduce metabolic functions, and assume a body temperature close to that of their surroundings (2–7°C). These physiological adaptations and behaviors likely predispose bats to infection by *G. destructans* and consequent development of WNS. Because approximately half the bat species of the United States are obligate hibernators, another 19 species are at risk for infection by *G. destructans* if it spreads beyond its current range.

G. destructans colonizes the skin of bat muzzles, wings, and ears, then erodes the epidermis and invades the underlying skin and connective tissues. This pattern is distinctive and is more severe than that caused by typical transmissible dermatophytes. Although the disease was named for the characteristic white growth visible around an infected animal's nose, the primary site of infection is

the wing (Figure A4-3A). Gross damage to wing membranes such as depigmentation, holes, and tears are suggestive of WNS, but these lesions are nonspecific, and histopathologic examination is necessary to diagnose the disease.

Specifically, fungal invasion of wing membranes ranges from characteristic cup-like epidermal erosions filled with fungal hyphae to ulceration and invasion of underlying connective tissue, with fungal invasion sometimes spanning the full thickness of the wing membrane (Figure A4-3B). Fungal hyphae can also fill hair follicles and destroy skin glands and local connective tissue. Bat wings play an important role in the pathogenesis of WNS by providing a large surface area for the fungus to colonize. Once infected, the thin layer of skin that composes the bat wing is vulnerable to damage that may catastrophically disrupt homeostasis during hibernation.

In North America, bat hibernacula range in temperature from approximately 2–14°C, temperatures all permissive to growth of *G. destructans*. Within this temperature range, *G. destructans* exhibits increasing growth rates with increasing temperature (Figure A4-4), but the fungus does not grow at temperatures of approximately 20°C or higher. This temperature sensitivity helps to explain why WNS is observed only among hibernating or recently emerged bats and why the disease is not diagnosed in bats during their active season when body temperatures are consistently elevated above those permissive to growth of *G. destructans*.

Looking for Other Host and Environmental Susceptibility Factors

Hosts with impaired immune functions tend to be susceptible to opportunistic fungi in their environments. Guided by this concept, some investigators suspected that insults such as exposure to environmental contaminants or infections by viral pathogens compromised bat immunity and made them vulnerable to *G. destructans*. However, neither contaminant exposure nor viral coinfections can be consistently identified in bats infected with that fungus.

Hibernating bats with WNS generally do not exhibit signs of an inflammatory response. However, severe inflammation typifies fungal skin infections of bats aroused from hibernation, providing evidence that such animals are not immunocompromised. Although studies of bat immune functions are in their infancy, studies of other mammalian species indicate that their immune functions are naturally suppressed during hibernation. Thus, rather than suggesting immune-function impairment, the lack of inflammatory response to fungal infection by hibernating bats may reflect an immune suppression that is part of hibernation physiology.

In addition, the body temperature of hibernating bats drops dramatically, providing another vulnerability to infection by *G. destructans*. Fatal fungal diseases are relatively rare among endothermic, or warm-blooded, animals because their tissues are too warm to support the growth of most fungal species. However,



FIGURE A4-3 (A) Three little brown bats (*Myotis lucifugus*) photographed by Alan Hicks (New York State Department of Environmental Conservation) in Graphite Mine, New York, in November, 2008. Note the white fungus colonizing the muzzles and nostrils of all three bats. Also note the extensive fungal colonization of the skin of the ears and wings of the bat pictured on the right; (B) Periodic acid-Schiff (PAS) stained microscopic section of wing membrane from a little brown bat with white-nose syndrome collected in Pennsylvania in February, 2009. Dense colonies of fungal hyphae erode skin and fill the cup-shaped depressions (arrow). Ulceration of epidermis with penetration and replacement of subcutaneous tissue (arrow heads) dramatically alters the integrity of wing membrane. Bar = 25 μ m.

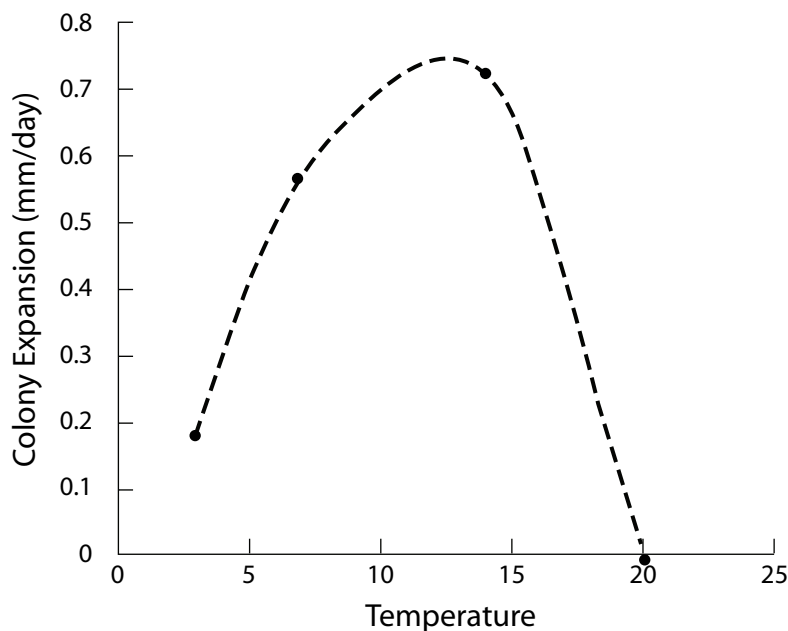


FIGURE A4-4 Colony expansion rates of *Geomyces destructans* when grown on corn-meal agar at 3, 7, 14, and 20°C. The trend line estimates colony expansion rates at temperatures ranging from 3–20°C.

fungi are more apt to cause fatal diseases in ectothermic, or cold-blooded, organisms such as insects, fish, amphibians, and plants. Bats and other mammals that hibernate are unique in that they are warm-blooded when metabolically active, but cold-blooded during hibernation—a period when their metabolism and body temperatures are dramatically suppressed. Although lowered body temperatures may predispose torpid bats to infection by *G. destructans*, the mechanism enabling this specific fungus to be a pathogen for bats while other cave-associated fungi remain innocuous is not known.

How *G. destructans* kills bats is under active investigation. One possibility is that fungal infection disrupts how bats behave while hibernating, leading to more frequent or longer arousals from torpor and thus accelerating usage of fat reserves. However, fat depletion is not consistently observed among all bats with WNS. Infected bats also may exhibit other aberrant behaviors midway through the hibernation season, such as shifting from thermally stable roost sites deep within hibernacula to areas with more variable temperatures near entrances.

Sometimes, they depart early from hibernacula. Thus, exposure to cold could account for some WNS-associated mortality.

Further, fungal damage to wing membranes, which can account for more than 85% of the total surface area of a bat, may increase fatality rates. In addition to the key role that wings play in flight, wing membrane integrity is essential for maintaining water balance, temperature, blood circulation, and cutaneous respiration. Disrupting any of these functions could increase WNS mortality rates.

As with so many other diseases, the environment affects the progress and transmission of WNS. Some pathogenic fungi such as *Histoplasma capsulatum*, *Cryptococcus* spp., and *Batrachochytrium dendrobatidis* can persist in the environment without an animal host for survival. This independence contrasts with host-requiring viruses or other pathogens for which transmission dynamics tend to moderate as infected hosts are removed from a population. *G. destructans* likely does not require bat hosts to survive and can persist in caves by exploiting other nutrients.

The cool and humid conditions of underground hibernacula provide ideal environmental conditions for *G. destructans* or other fungal growth. While most *G. destructans* isolates were cultured from skin or fur of bats collected in or near underground hibernacula during winter, DNA from the same fungus is found in soil samples from several hibernacula that harbor WNS-infected bats in the northeastern US. Also, *G. destructans* has been cultured from soil samples from hibernacula in three states where WNS occurs, supporting the hypothesis that bat hibernacula are reservoirs for this pathogen and that bats, humans, or fomites may transport *G. destructans* between hibernacula. How temperature and humidity differences among hibernacula influence *G. destructans* and WNS is not known.

Uncertainties about WNS Emergence

What caused WNS to emerge in a North American cave during the winter of 2005 to 2006? Bats with clinical signs consistent with WNS were first observed in Howes Cave, a hibernaculum connected to a popular North American show cave. Because of its high human traffic, a tourist might have inadvertently introduced *G. destructans* at this site.

Europe might be the source for the fungus causing WNS. Reports dating back several decades describe hibernating bats in Germany with white muzzles resembling bats with WNS in North America. Recent culture and PCR surveys indicate that *G. destructans* is widespread in Europe, including among hibernating bats in hibernacula in the Czech Republic, France, Germany, Hungary, Slovakia, and Switzerland. Unlike in North America, however, mortality rates and population declines remain normal among European bat species. This sharp contrast between disease manifestation among bats in Europe and North America provides an opportunity to investigate how bat species may differ in terms of their susceptibilities to fungal infection, continental variability among fungal strains, and the influence of environmental conditions and bat behavior on this fungal disease.

Challenges in Managing WNS, Conserving Bat Populations

Bat conservation efforts have historically focused mainly on reducing human causes of bat mortality, including habitat destruction, detrimental intrusions into roosts, and intentional extermination of colonies. Bat census figures prior to the emergence of WNS in North America indicate many populations of cave-hibernating bats were stable or increasing. However, the current WNS outbreak brings an even more serious threat to bat populations of North America, confronting biologists with a new set of conservation and management challenges.

Mitigating diseases in free-ranging wildlife populations requires very different approaches from those applied in agriculture for domestic animals. Once established, diseases in free-ranging wildlife are rarely, if ever, eradicated. Biologists trying to manage WNS within bat populations face multiple challenges, including the need to deal with numerous host species, long-distance migrations of infected hosts, poor access to some host populations, impracticalities associated with treating individual wild animals, infected hosts that are sensitive to being disturbed and that inhabit fragile ecosystems, and environmental persistence of the pathogen.

The guiding principle for physicians and veterinarians, “first, do no harm,” will help to prevent WNS management efforts from having unintended adverse consequences. For example: depopulating an infected colony would not be effective unless all infectious animals are removed and all hibernacula used by the population are decontaminated—conditions unlikely to be achieved among free-ranging wildlife; using disinfectants to decontaminate hibernacula could have toxic effects on other organisms reliant on those environments; treating individual bats with antifungal agents is labor intensive, is not self-sustaining, and could be toxic for treated animals or their symbionts; and careless intervention could disrupt natural selective processes that might yield behaviorally or immunologically resistant bats.

However, “first, do no harm” does not mean “do nothing.” State and federal agencies already are taking measures to combat WNS, including closing caves and mandating decontamination procedures. Such steps are intended to prevent people from disturbing hibernating bats and to reduce the chance that intruding humans will transfer *G. destructans* from one hibernaculum to another. For example, taking a proactive approach prior to the appearance of WNS, state wildlife officials in Wisconsin conferred threatened status on four cave bat species that hibernate within its borders and designated *G. destructans* a prohibited invasive species providing state resource managers with legal authorities to take disease management actions.

Since the first description of *G. destructans* in 2008, its genome has been sequenced, and WNS pathology has been more fully defined. Additionally, hibernacula are being surveyed internationally, and ongoing analyses are revealing much about the biodiversity of fungi associated with bat hibernacula. With these and other advances in understanding WNS, opportunities will arise to better

manage the disease cycle. The sudden and unexpected emergence of WNS exemplifies the importance of monitoring, investigating, and responding to emerging wildlife diseases and the ecological and societal threats that they present.

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A5

**MAMMALIAN ENDOTHERMY OPTIMALLY
RESTRICTS FUNGI AND METABOLIC COSTS^{18,19}***Aviv Bergman²⁰ and Arturo Casadevall²¹***Abstract**

Endothermy and homeothermy are mammalian characteristics whose evolutionary origins are poorly understood. Given that fungal species rapidly lose their capacity for growth above ambient temperatures, we have proposed that mammalian endothermy enhances fitness by creating exclusionary thermal zones that protect against fungal disease. According to this view, the relative paucity of invasive fungal diseases in immunologically intact mammals relative to other infectious diseases would reflect an inability of most fungal species to establish themselves in a mammalian host. In this study, that hypothesis was tested by modeling the fitness increase with temperature versus its metabolic costs. We analyzed the tradeoff involved between the costs of the excess metabolic rates required to maintain a body temperature and the benefit gained by creating a thermal exclusion zone that protects against environmental microbes such as fungi. The result yields an optimum at 36.7°C, which closely approximates mammalian body temperatures. This calculation is consistent with and supportive of the notion that an intrinsic thermally based resistance against fungal diseases could have contributed to the success of mammals in the Tertiary relative to that of other vertebrates.

Importance

Mammals are characterized by both maintaining and closely regulating high body temperatures, processes that are known as endothermy and homeothermy,

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respectively. The mammalian lifestyle is energy intensive and costly. The evolutionary mechanisms responsible for the emergence and success of these mammalian characteristics are not understood. This work suggests that high mammalian temperatures represent optima in the tradeoff between metabolic costs and the increased fitness that comes with resistance to fungal diseases.

Endothermy and homeothermy are fundamental aspects of mammalian physiology whose evolutionary origin remains poorly understood. Although many explanations have been suggested for the origins of endothermy and homeothermy, none are fully satisfactory given their high metabolic costs (Kemp, 2008; Ruben, 1995). Furthermore, the factors responsible for the mammalian set point remain unknown, posing the additional question of why mammals are so hot. Recently, the observation that fungal diseases are common in plants and insects but rare in mammals, combined with the thermal susceptibility of fungi, led to the proposal that mammalian endothermy and homeothermy create a thermal exclusionary zone that protects mammals against mycoses (Robert and Casadevall, 2009). Endothermy was also suggested to have provided a fitness advantage in the fungal bloom that followed the end of the Cretaceous such that it could have contributed to the success of mammals in the Tertiary (Casadevall, 2005; Robert and Casadevall, 2009).

Assuming that a relationship exists between endothermy and reduced susceptibility to certain classes of microbes, we hypothesized a tradeoff relationship whereby the high costs of endothermy were mitigated by protection against infectious diseases. In other words, we posited that increases in body temperature would protect against microbes by creating a thermal exclusionary zone but that such increases would be increasingly costly with regard to metabolic rates as the host body temperature diverged from ambient temperatures. Given that there is robust information on fungal thermal tolerances (Robert and Casadevall, 2009), we decided to test this hypothesis by attempting to identify body temperatures that confer maximal fitness for certain metabolic rates.

To address this question, we propose a first-order model wherein a tradeoff exists between the excess metabolic rates required to maintain a body temperature, T , and the benefit gained by protection against deleterious microbes because of the creation of a thermal exclusion zone. Metabolism, the exchange of energy between the organism and its environment, as well as the transformation of that energy to material within an organism, is affected by two main factors, body mass, M , and body temperature, T . Due to the fractal nature of transport networks, that is, vessel architecture and branching (Gillooly et al., 2001; Savage et al., 2008), over ontogeny, the resting metabolic rate, B_{rest} , scales with body mass, m , as $B_{\text{rest}} = B_0 m^{3/4}$, where B_0 is a normalization constant for a given taxon. Also, the normalization coefficient, B_0 , exponentially increases with body temperature $B_0 \sim e^{-E_0/KT}$, where E_0 is the average activation energy for the rate-limiting enzyme-catalyzed biochemical reactions of metabolism (ca. 0.65 eV), K is Boltzmann's constant ($8.62 \times 10^{-5} \text{ eV/K}$), and T is body temperature (Brown et al., 2004;

Gillooly et al., 2001). The scaling relationship between resting metabolic rate and body mass, $\propto m^{3/4}$, has been predicted from allometric theories and supported by data on a diverse set of organisms, including mammals, birds, fish, and mollusks (Brody, 1964; Moses et al., 2008; Savage et al., 2004; West et al., 1997). As can be seen from the formulas above, body temperature affects the metabolic rate through its effects on rates of biochemical reaction kinetics according to Boltzmann's factor, $e^{-E/kT}$, where T is measured in kelvins (absolute temperature). The resting metabolic rate, B_{rest} , is proportional to the product of these two effects and again has been shown to be well approximated, within a biologically relevant temperature range (0°C to 40°C), as $B(T) \propto e^{-E/kT} m^{3/4}$ (Gillooly, 2001). The first part of our analysis examined the excess cost for an organism of body mass m to maintain a body temperature T (assuming no dependence of body mass on temperature).

In the second part of our analysis, the benefit, noted here as $F(T)$, is calculated as the reduction in the number of fungal species capable of infecting a host; this number is reduced approximately by $s \approx 6\%$ for every degree Celsius in the temperature range of 27°C to 40°C (Robert and Casadevall, 2009). The increased benefit of the successive elimination of fungal species can thus be expressed as $F(T) \propto F_0[1 - (1 - s)^T]$, where F_0 is a constant scaling factor. The quantity $W(T) = F(T)/B(T)$ can represent the balance between cost and benefit; thus, $W(T)$ can be viewed as the total fitness of an organism as a function of its body temperature. Within the biologically relevant temperature range, the proposed fitness measure reaches its maximum at approximately 37°C (Fig. A5-1). Note that in this formulation, the optimal body temperature, where $W(T)$ attains its maximum value, does not depend on the organism's body mass. Furthermore, the one parameter that is determined from biological observation is the reduction in the number of fungal species capable of infecting a host; thus, to determine our model's dependence on this parameter, we calculated the optimal temperature over a wide range of possible reduction percentages, i.e., 4% to 8%. In this range, the optimal temperature was found to remain in a tight range of less than 2°C, from 37.7°C to 35.9°C, respectively, which is still within the biologically relevant range of mammalian body temperatures. The insensitivity of the model to its only parameter further strengthens our hypothesis.

In summary, we present a minimal, parsimonious model to account for the cost of maintaining a high body temperature in mammalian organisms. A body temperature of 36.7°C maximizes fitness by restricting the growth of most fungal species relative to its metabolic cost. Our model suggests that no additional elaborations are required to explain the evolution of endothermy other than the tradeoff between protection against environmentally acquired microbial diseases and the cost of metabolism. Although we cannot rule out the possibility that this body temperature optimum arose by some remarkable coincidence, we think this highly unlikely because it emerges from considering two unrelated processes, fungal thermal tolerance and mammalian metabolic costs. Nonethe-

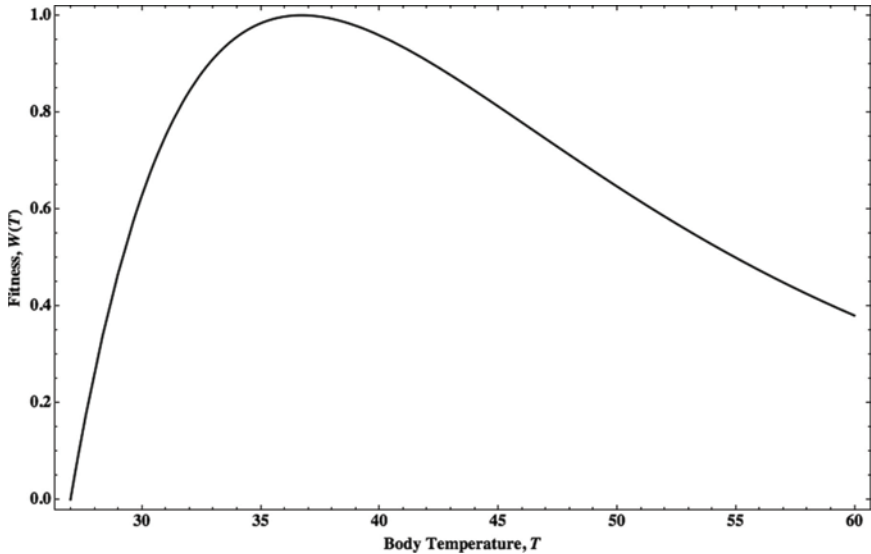


FIGURE A5-1 Organism fitness as a function of body temperature. We normalized fitness, $W(T)$, to attain a maximum value of 1 and plotted body temperature in degrees Celsius over a range of 27°C to 60°C. Fitness reaches a maximum value at $W_{\text{MAX}}(T) \approx 36.7^\circ\text{C}$.

less, we acknowledge that similar temperature optima might emerge from other considerations. For example, the specific heat capacity of water has a minimum at 36°C, and if the efficiency of metabolic processes is related to heat capacity, then using this parameter as the optimality criterion may result in a similar range of solutions. Nevertheless, we note the internal consistency in the theme that fungal diseases are rare in immunologically intact mammals and the tradeoff between increased fitness and metabolic costs closely approximates mammalian body temperatures.

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A6

VERTEBRATE ENDOTHERMY RESTRICTS MOST FUNGI AS POTENTIAL PATHOGENS^{22,23}

Vincent A. Robert²⁴ and Arturo Casadevall²⁵

The paucity of fungal diseases in mammals relative to insects, amphibians, and plants is puzzling. We analyzed the thermal tolerance of 4802 fungal strains from 144 genera and found that most cannot grow at mammalian temperatures. Fungi from insects and mammals had greater thermal tolerances than did isolates from soils and plants. Every 1°C increase in the 30°C–40°C range excluded an

²² Vincent A. Robert and Arturo Casadevall, “A Vertebrate Endothermy Restricts Most Fungi as Potential Pathogens”, *Journal of Infectious Diseases*, 2009, Vol. 200, Iss. 10, pp. 1623–1626. Reprinted by permission of Oxford University Press.

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additional 6% of fungal isolates, implying that fever could significantly increase the thermal exclusion zone. Mammalian endothermy and homeothermy are potent nonspecific defenses against most fungi that could have provided a strong evolutionary survival advantage against fungal diseases.

Of the 1.5 million fungal species, only a few hundred are pathogenic to mammals (Kwon-Chung and Bennett, 1992). Fungal diseases in mammals often reflect impaired immune function, and fungi did not emerge as major pathogens for humans until the late 20th century. For example, candidiasis was uncommon until the 1950s, when thrush was associated with the introduction of antibiotics that disrupted bacterial flora. Similarly, diseases such as cryptococcosis, aspergillosis, and histoplasmosis were rare until recently, when their prevalence increased with the human immunodeficiency virus epidemic and the development of immunosuppressive therapies. In contrast, the number of fungal species pathogenic to plants and insects is estimated to be 270,000 and 50,000, respectively (Hawksworth and Rossman, 1997). Amphibians are particularly vulnerable to certain fungal infections, as evidenced by the current catastrophic epidemic of chytridiomycosis in frogs.

The resistance of mammals with intact immune systems to systemic fungal diseases, coupled with their endothermic and homeothermic lifestyles, suggested that these costly physiological adaptations were evolutionarily selected because they conferred a survival advantage by protecting against environmental pathogens (Casadevall, 2005). However, testing this hypothesis was difficult because knowledge of fungal thermal tolerance is largely anecdotal. Consequently, we evaluated the thermal growth tolerances of fungal species in a reference collection and compared them to mammalian temperatures.

Methods

A total of 4802 fungal strains belonging to 144 genera in the Centraalbureau voor Schimmelcultures (Utrecht) collection were tested for growth at 4°C, 12°C, 15°C, 18°C, 21°C, 25°C, 30°C, 35°C, 37°C, 40°C, 42°C, and 45°C. Strains were grown for times ranging from a few days to a few weeks on the most suitable medium, generally glucose–peptone–yeast extract agar, potato–dextrose agar, or yeast extract–malt extract agar. Growth was considered positive when a colony was visible without magnification. The strain set included Ascomycetes and Basidiomycetes but excluded Zygomycetes, which is not in the yeast database.

The culture deposit records were reviewed to identify the isolation source. Fungi isolated from flowers, grains, and herbal exudates were grouped under plant isolates. Animal isolates were classified depending on whether they originated from endothermic (mammals and birds) or ectothermic (insects, nematodes, fishes, and crustaceans) species. Another group comprised isolates from nonliving environmental sources, which included predominantly soils; this group is referred to as soil isolates. These groups were compared for thermal tolerance at 2 tem-

peratures, 25°C and 37°C, which reflect ambient and mammalian temperatures, respectively.

To test the significance of the difference in growth patterns between fungal strains isolated from different groups, we calculated the test statistics

$$z = (p^1 - p^2) / \sqrt{P \times (1 - P) \times (1/n^1 + 1/n^2)},$$

where p^1 and p^2 are the observed sample proportions for each group of fungal strains at a given temperature, n^1 and n^2 are the size of the 2 groups under comparison, and

$$P = (p^1 \times n^1 + p^2 \times n^2) / (n^1 + n^2).$$

The statistic z was assumed to be distributed normally. The 2-tailed probability from the absolute z score to infinity on both tails of the distribution was calculated (<http://www.danielsoper.com/statcalc/calc21.aspx>) and confirmed using the NORMSDIST function in Excel (Microsoft) to assess the significance of differences in growth between groups at different temperatures.

Results and Discussion

Knowledge of fungal thermal tolerance is limited to a few species because the subject has not been systematically studied. In fact, such studies may be very difficult to do, and a comprehensive prospective study of fungal thermal tolerance would require a gargantuan effort. However, culture collections provide an attractive alternative for initial explorations of this subject. Culture collections store and maintain fungal strains and record basic nutritional needs and temperature tolerances. This information, when accessed and analyzed with bioinformatics tools, provides a useful starting point for the analysis of fungal thermal tolerances.

Our results show that most strains grew well in the 12°C–30°C range, but there was a rapid decline in thermal tolerance at temperatures >35°C (Figure A6-1). A plot of the fraction of fungal strain that grew versus temperature in the 30°C–42°C range revealed a linear relationship with an equation of $y = -0.0166x + 2.7911$, such that for every 1° increase in temperature >30°C, ~6% fewer strains could grow.

For 3020 strains, there was information on both source isolation and temperature tolerance. This group included isolates from the environment (primarily soils), plants, ectothermic animals, and endothermic animals. The majority of these isolates grew at 25°C regardless of their source (Table A6-1). Nevertheless, the proportion growing at 25°C was significantly greater for isolates recovered from living hosts than from soils, irrespective of whether the hosts were ectothermic plants and animals or endothermic animals. At 37°C, the proportion of

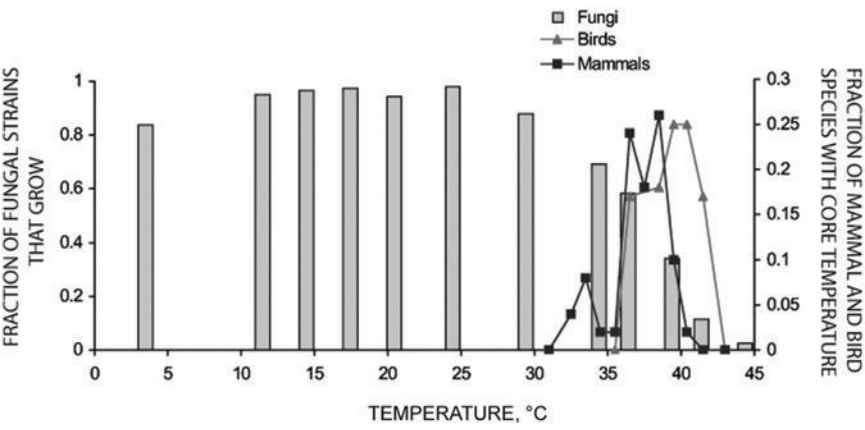


FIGURE A6-1 Frequency histogram of thermal growth tolerance for 4802 fungal strains (bars). Lines connect percentages for 49 mammalian (blue) and 12 bird (red) species core temperatures. Obtained from McNab (1970).

TABLE A6-1 Growth Tolerances for Fungi from Soils, Animals, and Plants at 2 Temperatures

Origin, host type	Isolate Growth			Total	<i>P</i> values ^b		
	Yes	No	Unknown ^a		<i>P</i> 1	<i>P</i> 2	<i>P</i> 3
at 25°C							
Soils, NA	657	42	7	706			
Plant, ectotherm	1108	30	5	1143	<.001		
Animal							
Ectotherm	490	0	6	496	<.001	.29	
Endotherm	661	5	9	675	<.001	.214	.263
at 37°C							
Soils, NA	146	535	15	706			
Plant, ectotherm	304	871	22	1143	.292		
Animal							
Ectotherm	193	284	19	496	<.001	.004	
Endotherm	466	202	7	605	<.001	<.001	<.001

NOTE. NA, not applicable

^a Refers to a small no. of isolates for which the temperature growth data was not complete.

^b P1 refers to the comparison of isolates from soils, P2 refers to the comparison versus plant isolates, and P3 refers to the comparison between isolates from ectothermic and endothermic animals.

fungi that grew was much higher for isolates from endothermic animals than from ectothermic animals. The proportions of Ascomycetes and Basidiomycetes fungi in each group were comparable, except for ectothermic hosts, which yielded predominantly Ascomycetes fungi.

Isolates from ectothermic hosts (such as plants and insects) were significantly more thermotolerant than isolates from soils. A significantly greater percentage of fungal strains from insects grew at 37°C relative to those recovered from plants, possibly reflecting the fact that insects can increase their temperature through behavioral fevers that increase survival after fungal infection (Thomas and Blandford, 2003). However, this explanation is unlikely to apply to plants, which have much lower metabolic rates. Since thermal tolerance must be associated with numerous metabolic changes that mitigate fungal damage, the association between greater thermotolerance and plant pathogenicity could mirror adaptation to survival in a host with potent antifungal defenses, raising the tantalizing possibility that selection pressures by virulence may contribute to thermal stability and vice versa. In this regard, we note that Hsp90 orchestrates morphogenesis in *Candida albicans* (Shapiro et al., 2009), thus providing a molecular association for heat shock and a virulence-related phenotype that may be conserved in other fungi.

A survey of the fungal genera represented in our sample collection revealed differences in the percentage of isolates capable of growth at 37°C. All genera studied included some thermotolerant species, as defined by their ability to grow at 37°C, but there were large differences in the percentage of species within each genera. Thermotolerant genera included those from both Ascomycetes and Basidiomycetes, but basidiomycetous genera were disproportionately more common among the thermotolerant genera ($P < .001$, Fisher exact test). The strains grouped within the sexually related basidiomycetous genera *Filobasidiella* (a telemorph of *Cryptococcus*) and *Cryptococcus* (an anamorph of *Filobasidiella*) included comparable numbers of thermotolerant species (61% among 116 strains and 53% among 287 strains, respectively). These data suggest an association between phylogeny and thermotolerance.

The capacity for thermotolerance was interspersed among Ascomycetes and Basidiomycetes, suggesting that it may have emerged independently several times in evolution. Alternatively, thermotolerance may be an ancient fungal trait that was lost by those species that cannot grow at 37°C. In this regard, we note that the climate for much of Earth's history was much warmer than in recent geologic epochs, having cooled by ~5°C during the Eocene-Oligocene transition ~34 million years ago (Liu et al., 2009). The fact that thermotolerance is a complex trait that can be lost by a single mutation, as demonstrated by laboratory-generated temperature-sensitive mutants, makes the explanation of a retained phenotype attractive.

Our results may be relevant to the ongoing debate on the origin and function of endothermy, homeothermy, and fever, each a major unsolved problem in vertebrate physiology (Kemp, 2008; Ruben, 1995). There is no consensus as to

why mammals have adopted such an energetically costly lifestyle. Endothermy is associated with certain metabolic benefits and thermodynamic efficiency, but these benefits come at a high cost since endothermic vertebrates require ~10 times more oxygen to support metabolism than do ectothermic vertebrates (Ruben, 1995). Our analysis suggests that part of the cost is mitigated by the creation of a thermal exclusionary zone that can protect against environmental microbes. Given the high metabolic cost of endothermy, the core temperatures of individual mammal and bird species are likely to be a compromise between its benefits and costs. If endothermy was selected for protection against infectious disease, then a case could be made that endothermy preceded homeothermy. Similarly, if one considers fever as a mechanism to extend the thermal exclusionary zone against environmental microbes such as fungi, increases in temperature of only 1°–3° can significantly reduce the proportion of such microbes that can inhabit the host.

The benefits of endothermy and homeothermy in protection against microbes do not appear to have been previously considered as mechanisms for evolutionary selection, possibly because most of the viral and bacterial diseases that currently plague animals are often acquired from other warm hosts, and these necessarily involve thermotolerant microbes. However, the perspective is very different when one focuses on environmentally acquired microbes and the fungi in particular. Pathogenic microbes are a very small subset of the total terrestrial microbial flora, and these can be divided as to whether they are acquired from other hosts or directly from the environment (Casadevall and Pirofski, 2007). For mammals, pathogenic microbes acquired from other hosts are usually adapted to mammalian temperatures, but microbes acquired directly from the environment would not be subject to such selection pressures. Hence, the potential benefit of endothermy and homeothermy to host defense may become apparent only when one considers the entire microbiota and that subset of pathogenic microbes that is acquired directly from the environment. In support of this notion, we note that bats become susceptible to a cold-loving fungus when hibernation greatly reduces their body temperatures (Bleher et al., 2009) and that primitive mammals (such as the egg-laying platypus, which has a body temperature of 32°C) are susceptible to fungal diseases (Obendorf et al., 1993). An epidemiological observation consistent with the protective function of endothermy comes from the observation that serotype D *Cryptococcus neoformans* are less thermotolerant (Martinez et al., 2001) than other varieties and are associated with cutaneous cryptococcosis (Dromer et al., 1996). Experimental support for the notion that endothermy restricts fungal infection comes from the observation that rabbits, which have core temperatures of 38°C–39°C, are notoriously resistant to cryptococcosis, and infection can be induced only in cooler organs, such as testes (Perfect et al., 1980). However, the same system also shows that mammalian immune systems also make a decisive contribution to host defense against fungi since systemic cryptococcosis can be induced in rabbits after corticosteroid administration (Perfect et al., 1980).

This study reflects the power of a bioinformatics analysis of archival data

from culture collection, which allows comparison of temperature growth data on thousands of isolates. However, there are certain limitations that should be considered in evaluating the data. Strains from plants and insects were disproportionately represented in the collection, and this may introduce certain biases. The relative paucity or absence of strains from certain sources and taxonomic groups could contribute to bias in the statistical analysis. For example, there were relatively few isolates from birds and ectothermic animals other than insects, no Zygomycetes fungi, and only a few filamentous fungi. Furthermore, the catalogued information was insufficiently detailed to distinguish between skin and systemic isolates from endotherms, which could differ in thermotolerance.

The discovery of fossilized fungal proliferation at the Cretaceous-Tertiary boundary was proposed to contribute to extinction events at the end of the Cretaceous epoch that replaced reptiles with mammals as the dominant large animals (Casadevall, 2005). Thermal tolerance is a necessary, but not sufficient, characteristic of microbes being capable of causing invasive disease in mammals. Since thermal tolerance almost certainly involves many genes and biochemical processes, it is unlikely that this trait can be rapidly acquired by any one microbial species. Consequently, new human pathogenic fungi are likely to emerge from genera that are already tolerant to higher temperatures; such species may warrant special attention given likely climatic changes in the years ahead that could alter patterns of fungal prevalence.

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A7

SURVEILLANCE FOR EMERGING DISEASES IN WILDLIFE

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Impact of Fungal Diseases on Wildlife

At this meeting, a number of presenters discussed the role of specific fungal diseases in rapid declines and even extinctions of wildlife. Getting an accurate measure of the impact of a pathogen or group of pathogens on wildlife is notoriously difficult. First, wildlife populations undergo often dramatic shifts that are difficult to distinguish from the effects of an outbreak. These may be seasonal or interannual, and occur in response to long-term climatic fluctuations, variation in predator–prey cycles, or a range of other difficult-to-measure factors (Daszak et al., 2005; Pechmann et al., 1991). Second, outbreaks of disease in wildlife may cause significant mortality, but these events may be difficult to detect due to rapid scavenging or decay of carcasses. Even when carcasses are found, they may be too decayed to conduct proper pathological investigations. Third, despite a range of infectious agents linked to recent declines, these are relatively new to ecologists and wildlife managers, so that die-offs are often attributed to other factors, and diseases may not be examined. Despite these and other issues, emerging diseases—indeed, emerging *fungal* diseases—have been shown to cause significant population declines and even extinctions in wildlife (Daszak and Cunningham, 1999; Frick et al., 2010; Schloegel et al., 2006).

The emerging fungal disease chytridiomycosis is a good example of this trend. As discussed elsewhere in this report, it is caused by the fungal pathogen *Batrachochytrium dendrobatidis*, which infects the keratin-rich cells on the skin of adult amphibians (Kilpatrick et al., 2010). This disease was discovered in the 1990s and associated with significant population declines in Australia and Central America (Berger et al., 1998). Substantial support from other studies shows it is the major cause of global amphibian declines (Crawford et al., 2010; Lips et al., 2006; Skerratt et al., 2007). However, when this disease was first reported, a debate took place in the literature over whether amphibians were undergoing

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population declines, or simple fluctuations (Blaustein, 1994). Debate continued on the importance of chytridiomycosis and other factors. The ecological community took about a decade to accept the role of disease in these wild animals as important enough to call for large-scale global action to prevent further spread (Mendelson et al., 2006).

Despite these issues, reports of emerging diseases affecting wildlife populations have grown rapidly (Aguirre and Tabor, 2008; Cunningham, 2005; Daszak et al., 2000; Deem et al., 2001; Nettles, 1996; Wildlife and emerging disease, 2009; Williams et al., 2002). These include diseases that have caused a number of high-profile declines, such as mycoplasmal conjunctivitis of house finches in the United States (Fischer et al., 1997); trichomonosis in declining birds in the United Kingdom (Robinson et al., 2010); chronic wasting disease of cervids in the United States (Miller and Williams, 2004; Sigurdson, 2008); and white-nose syndrome (Blehert et al., 2009) or geomyocosis (Chaturvedi and Chaturvedi, 2011) of bats. Among these are a surprising number of fungal diseases, which often have a high impact. In humans, fungal pathogens do not represent a major cause of emerging diseases.

Analysis of a global database of emerging pathogens (Jones et al., 2008) suggests that fungi are responsible for only 5.9 percent of the emerging infectious diseases of people in the past four decades (Figure A7-1). Yet in wildlife, fungi have been implicated in global declines of amphibians, leading to extinction of species (Schloegel et al., 2006), multistate declines of bat populations (Frick et al., 2010), the near extinction of the Florida *Torreya* tree (Schwartz et al., 1995, 2000), and the collapse of eel grass beds, leading to global extinction of the eel grass limpet *Lottia albicans* (Carlton, 1993; Carlton et al., 1991).

Challenges in the Surveillance of Wildlife for Fungal and Other Pathogens

The emergence of so many high-impact diseases in wildlife and the role of wildlife as reservoirs for human emerging infectious diseases, or EIDs (Mahy and Brown, 2000; Taylor et al., 2001), have led to expanding efforts in surveillance of wildlife populations, both for new pathogens of human significance and for potential EIDs affecting wildlife. However, a number of important factors hinder this strategy. Here, we highlight the two most important challenges to effective global surveillance in wildlife, as well as review some approaches to address these challenges; and put them into context for emerging pathogens of humans and wildlife, and for fungal diseases in particular.

Jurisdictional Problems in the Surveillance of Wildlife

The agencies, funding bodies, non-governmental organizations (NGOs), and researchers that normally work with wildlife populations are usually distinct from those involved in public health and agricultural diseases. For example, in

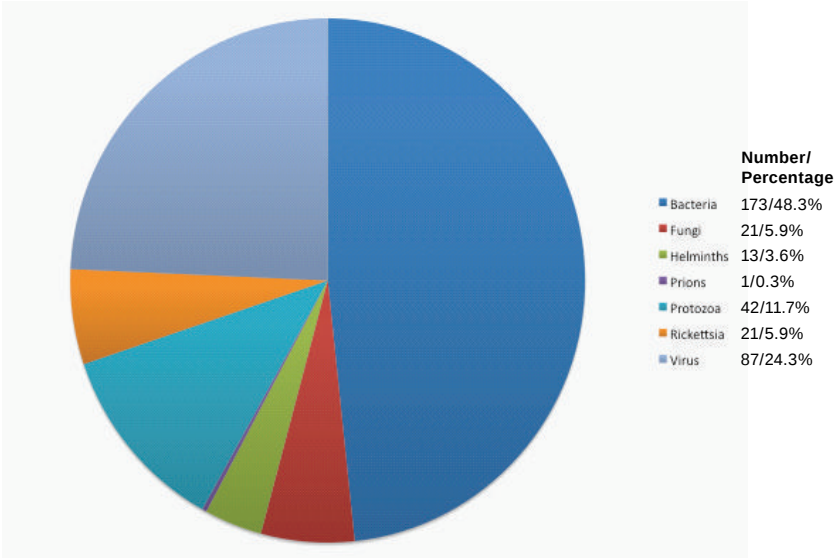


FIGURE A7-1 Proportion of emerging infectious diseases caused by different taxonomic groups of pathogens. Fungi are responsible for only 5.9 percent of emerging human disease, yet have a disproportionate impact on wildlife, with responsibility for a series of major population declines and extinctions. The data are taken from the database published in a recent global analysis of emerging infectious diseases of humans over the last four decades.
SOURCE: Adapted from Jones et al. (2008).

the United States, the U.S. Fish and Wildlife Service is the agency responsible for protecting wildlife, but if the threat is an emerging pathogen, this agency has little capacity for outbreak investigation and control. Similarly, the national agency responsible for funding ecological research in the United States is the National Science Foundation (NSF). The National Institutes of Health (NIH) oversees a broad range of issues, from infections to organ dysfunction to mental health and other areas. The U.S. Department of Agriculture oversees agricultural health. At the international scale, the intergovernmental agency to protect wildlife is the International Union for the Conservation of Nature (IUCN), whereas the global health agenda falls under the World Health Organization (WHO), agricultural health under the Food and Agriculture Organization (FAO), and trade-related disease issues under the World Organisation for Animal Health (OIE). This siloed approach is followed by many countries globally; they tend to have separate ministries for health, agriculture, trade, and environment/forestry/wildlife. These approaches work well until the threats to human health cross these jurisdictional boundaries. With emerging diseases, they have done so repeatedly. For example, the emergence of severe acute respiratory syndrome involved wildlife reservoir

species (Li et al., 2005), the national and international trade in hunted and farmed wildlife and livestock (Xu et al., 2004), and international travel and migration (Anderson et al., 2004). Likewise, the global emergence of amphibian chytridiomycosis has been linked to trade and climate change (Lips et al., 2008), and involves the medical industry (Weldon et al., 2004), the production of amphibians for food (Schloegel et al., 2009), and introduced or invasive species (Kilpatrick et al., 2010).

One simple approach to overcoming these challenges is to encourage cross-disciplinary, cross-agency collaboration. This approach to research and policy has been led by the fields of “conservation medicine” (Daszak et al., 2004), One Health (Karesh and Cook, 2005), and EcoHealth (Daszak, 2009; Wilcox and Daszak, 2006). In the United States, some efforts have successfully bridged the funding gap between NIH and NSF, notably the Ecology of Infectious Diseases program launched jointly by the NSF and the NIH John E. Fogarty International Center in 2000 (Scheiner and Rosenthal, 2006). Likewise, there is a unique U.S. federal agency with a specific remit to address with wildlife diseases, the National Wildlife Health Center (NWHC) (Fleischli et al., 2004; Skerratt et al., 2005). The NWHC has been conducting surveillance, monitoring, investigation, research, and response on wildlife diseases for 35 years, and is registered with the Centers for Disease Control and Prevention Select Agent Program, marking it as a laboratory of significant relevance to human and livestock as well as wildlife health. It has a sophisticated network of laboratories, including Biosecurity Level 3 biocontainment labs, necropsy suites, and isolation rooms. In addition, it publishes quarterly reports of mortality investigations, and acts as a national focal point for similar activities in universities and NGOs. At the intergovernmental scale, there has been a recent flurry of activity to bring together agencies around the One Health agenda, including formal links among the OIE, FAO, and WHO, which originated from their collaborative efforts to tackle avian influenza (Anderson et al., 2010). Additionally, wildlife health has two significant nuclei within the United Nations system.

First, in the IUCN, there is the Species Survival Commission Wildlife Health Specialist Group (http://www.iucn.org/about/work/programmes/species/about_ssc/specialist_groups/specialist_group_pprofiles/veterinary_sg_profile/), which has a network of more than 400 wildlife veterinarians and researchers globally. Second, the OIE has a Working Group on Wildlife Diseases (http://web.oie.int/wildlife/eng/en_wildlife.htm), which has operated for more than 15 years and advises the OIE on wildlife health issues.

These initiatives have begun to bring diverse disciplines together to understand the drivers and impacts of wildlife EIDs, and to conduct effective surveillance and control of wildlife as reservoirs for human EIDs. However, they could be improved significantly with some simple approaches. First, within the United States, the government has the capacity to form *interagency task forces* for specific issues that cross agency mandates. In a previous administration, the complex issue of amphibian declines was addressed with the formation of the Interagency

Taskforce on Amphibian Declines and Deformities. Similar task forces are likely to be useful to address the need for better surveillance of the wildlife trade for pathogens (Smith et al., 2009a), or the threat of white-nose syndrome in bats. Second, at a global scale, strengthening of laboratory capacity and personnel for wildlife diseases, and support for One Health approaches from the development community, could be extremely useful in fostering linkages among disparate ministries, universities, NGOs, and others. Recently, the U.S. Agency for International Development launched an Emerging Pandemic Threats program that specifically adopted a One Health approach to build capacity in the regions where emerging zoonoses most commonly originate (http://www.usaid.gov/our_work/global_health/home/News/ai_docs/ept_brochure.pdf) (Daszak, 2009). This includes specific collaboration among human and veterinary medical scientists; ministries of health, agriculture, and environment; and OIE, FAO, and WHO.

Limited Resources for Surveillance and Lack of Predictive Capacity

During the past decade, significant economic investment has been made in global surveillance for EIDs. Funding was provided by intergovernmental agencies, and national governments in particular, to counter the specific threat of H5N1 avian influenza. Although much of the surveillance and control efforts focused on Southeast Asia, the recent emergence of an H1N1 triple reassortant (Neumann et al., 2009; Smith et al., 2009b; WHO gets mixed reviews for H1N1 response, 2011) from a different region may have changed the public's perception of pandemic risk, and a shift of funding priorities. At the same time, the prominence of H5N1 in the media over the past decade and its inability so far to mount a pandemic heightens the perception of emerging diseases as difficult to predict or forecast. With limited global resources for pandemic prevention or control, predictive efforts that can help target resources are greatly needed.

Recent work has shown that analyzing past trends in disease emergence and correcting data for surveillance biases allows us a strategy to identify the regions most likely to propagate a new emerging zoonosis (Jones et al., 2008; King et al., 2006; Taylor et al., 2001; Woolhouse and Gowtage-Sequeria, 2005). These approaches may provide a way to geographically target surveillance efforts. They suggest that surveillance programs should target regions with high biodiversity and dense human populations, essentially in tropical and subtropical regions (Jones et al., 2008). This approach essentially analyzes the underlying drivers of emerging diseases, which are usually socioeconomic factors (e.g., travel, trade, agricultural changes) or environmental factors (e.g., climate, land use change). If we adapt this to fungal pathogens, we see that two drivers in particular are important—climate and trade. For human EIDs, analysis of the database in Jones et al. (2008) suggests that the majority of emerging fungal diseases were associated with HIV/AIDS or antimicrobial use. For plant EIDs, analyses suggest that agricultural trade and climate are the most important drivers of new emerging diseases (Anderson et al., 2004). With the limited work that has been conducted

on emerging fungal pathogens of wildlife, trade appears to be an important driver of emergence. For example, significant evidence points to the global amphibian trade for food as a major driver of the spread of the amphibian fungal disease chytridiomycosis (Schloegel et al., 2010). The similarity of U.S. isolates of *Geomyces destructans* (the causative agent of bat white-nose syndrome) with those from Europe and its continued spread as if from a focal point at a tourist cave in New York state strongly suggest anthropogenic introduction.

Two important recommendations emerge: First, targeting of wildlife trade routes, wet markets,²⁷ and international ports of entry for surveillance of wildlife may be particularly fruitful in identifying novel EIDs (Karesh et al., 2005; Pavlin et al., 2009; Smith et al., 2009a). Second, use of novel modeling approaches to geographically targeting wildlife for surveillance may be particularly useful for identifying novel pathogens. This “smart surveillance” approach (see Daszak, 2009) would help identify a pool of microbes harbored by a reservoir, some of which ultimately may become zoonotic under the right circumstances. Clearly, issues remain in that many microbes will be unable make the species jump to humans, so that distinguishing the truly potential zoonoses from these is not a simple task. However, conducting PCR-based surveillance in wildlife using conserved (or “degenerate”) primers for known pathogen groups would allow the targeting of novel agents related to known pathogens. This would enable a greater understanding of the risk of new zoonoses from each wildlife species examined, and would be a simple, cost-effective way to better evaluate the diversity of likely zoonoses. Diagnostic assays could then be developed for these pathogens, and used to screen people who live in close contact with the wildlife reservoirs.

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²⁷ Markets which sell live wildlife (often mixed with livestock) are called “Wetmarkets,” particularly with reference to Asia.

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A8

GEOGRAPHY, CLIMATE, DUST, AND DISEASE: EPIDEMIOLOGY OF VALLEY FEVER (COCCIDIOIDOMYCOSIS) AND WAYS IT MIGHT BE CONTROLLED

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Introduction

Human disease resulting from infection by *Coccidioides* spp. was first recognized late in the 19th century. Since then, with more information and changing demographics, our understanding of this problem and our perception of its importance has evolved in many ways (Galgiani, 2007). First thought of as a rare

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and always fatal illness, coccidioidomycosis later was appreciated as a common and frequently self-limited illness known as valley fever, in which only a small percentage of those affected suffered serious complications (Smith, 1940).

With U.S. military training within the endemic regions of California and Arizona during World War II, the potential of coccidioidomycosis as a significant problem affecting military readiness quickly became apparent, a problem that persists for the military into the present (Crum-Cianflone, 2007; Smith, 1958). With the rapid and extensive population expansion within south-central Arizona over the past decades, the impact of coccidioidomycosis has emerged from a rural to a much larger public health problem. With continued population growth within the central valley of California, in Mexico along the U.S. border, and in other endemic regions throughout the Western Hemisphere, it is expected that the numbers of infected persons will continue to increase. In addition to the medical problem for humans, a variety of other species, especially dogs, are susceptible to coccidioidomycosis and suffer considerable morbidity and mortality as a result (Shubitz, 2007).

A comprehensive assessment of coccidioidomycosis should address the fungus as it exists in the environment as well as how it creates medical problems. In both arenas, there are opportunities for better understanding which in turn could lead to improvements in public health. Hopefully, this article will be useful to call attention to where our knowledge is limited and how advances in those areas could benefit prevention and management of coccidioidal disease.

***Coccidioides* spp. in the Environment**

Our primary source for our understanding of the relative endemicity for regions within the United States is derived from studies conducted in the 1950s of coccidioidin skin-test prevalence of naval recruits from across the country (Palmer et al., 1957). These primarily include extensive portions of southern California, the lower deserts of Arizona, west Texas, and smaller areas of Utah, Nevada, and New Mexico. More recent information from California and Arizona (Hector et al., 2011), where coccidioidomycosis is a reportable infectious disease, is consistent with the earlier estimate. Coccidioidal infections are found in numerous other countries within the Western Hemisphere. What is known about these areas also suggests that the endemic distribution has been stable over the past several decades (Laniado-Laborin, 2007). On the other hand, the endemic regions in a longer time scale may not be constant. Recent archeological findings in Nebraska identified a Holocene bison dating back 8,500 years with coccidioidomycosis in a bone (Morrow, 2006). Also, models of wind pattern trends with global warming would predict increased westerly currents from the *Coccidioides*-endemic regions of west Texas toward the more populated portions of the state (Reheis and Rademaekers, 1997). Thus, with climate change we might see the endemic region expand.

Recent population studies of fungal isolates have identified genetic differences in isolates from patients in California (now classified as *Coccidioides immitis*) as compared to isolates from elsewhere (now classified as *Coccidioides posadasii*) (Burt et al., 1997; Fisher et al., 2000, 2001). This geographic separation is surprising, given the likelihood that coccidioidal spores should travel freely in and out of California, either by wind currents or as contaminants of trains, planes, or road vehicles. One possibility is that *C. immitis* is much better suited to its California niche than is *C. posadasii* and vice versa. Another explanation might relate to the biology of how soil becomes infected and endemic regions become established. For example, simply inoculating new soil may not be sufficient to create a new coccidioidal colony. Instead, a more complex interaction with rodents, plants, or other factors in the environment may be needed. Reinforcing this speculation is the sparse distribution of *Coccidioides* spp. within the environment. For example, in one systematic study in the central valley of California, only four genetically distinct isolates were found from 720 soil samples (Greene et al., 2000). Soil sampling in southern Arizona has also found isolates in a very small proportion of samples tested (Barker et al., 2010).

Researchers have known for some time that *Coccidioides* spp. are more prevalent in specimens from rodent burrows than from random subsurface soil (Elconin et al., 1957), although the reasons for this association remain unclear (Barker et al., 2010). In contrast, repeated sampling from a site known to be positive often yields positive results. One site identified as containing *Coccidioides* spp. in the 1960s (Converse and Reed, 1966) has again yielded *C. posadasii* half a century later (Personal communication, January 2011, M. J. Orbach). The sparse and stable nature of coccidioidal residence within the endemic regions affords an implication as to what factors are more likely to be associated with risk of exposure. Although there have been well-documented outbreaks associated with archeological and other dirt-disrupting sites (Pappagianis, 1983; Werner et al., 1972), in general occupational exposure does not appear to be a major risk factor (Kim et al., 2009). What might appear to be a paradox is actually consistent with the sparse prevalence of the fungus within the soil of even its most endemic regions, in which case much of the soil-disrupting activities occur at sites where the fungus is not present. In contrast, climate and especially wind patterns have been shown to affect seasonal incidence of infection (Comrie and Glueck, 2007; Hugenholz, 1957). Taken together, the evidence would suggest that simply length of endemic exposure rather than a specific activity constitutes the more dominant effect for risk of infection.

With our current understanding of *Coccidioides* spp. residing in the environment, it is not possible to predict whether it exists in a specific location with any degree of precision from a physical or chemical analysis of the soil. Furthermore, high-throughput methods do not exist for microbial detection of *Coccidioides* spp. in soil samples. These missing tools prevent us from identifying specific locations which, if disrupted, would likely create a release of fungal spores. If

methods were available to do this, methods for treating the soil exist that would minimize or prevent this exposure from happening. Advances in this area would have practical public health benefits.

Coccidioidomycosis: The Scope of the Problem

Statistics about newly diagnosed coccidioidomycosis from California and Arizona were expected to total more than 16,000 new infections in 2010 (Figure A8-1). The sharp increase in reported cases in Arizona in 2009 is the result of an administrative change by a single large clinical laboratory to report a more sensitive coccidioidal serologic test as indicative of a new infection when positive. However, for both states there has been increased disease activity in the last half of 2010 that is unexplained.

In Arizona, the Department of Health Services conducted a telephone questionnaire of 10 percent of newly identified patients in 2007. It provides a better understanding of the impact of this disease (Tsang et al., 2010). According to the survey, illness lasted for an average of 6 months; three quarters of employed

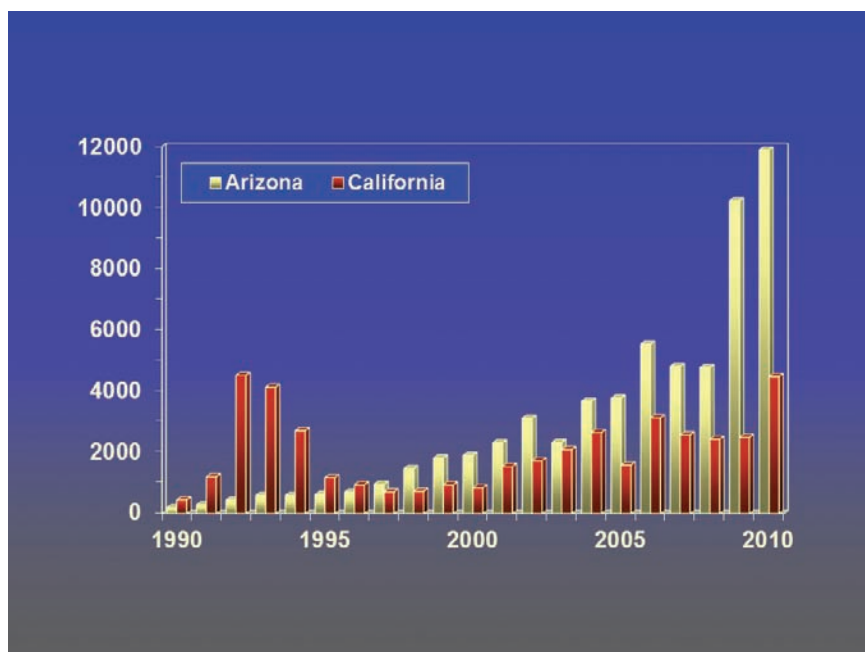


FIGURE A8-1 Annual cases of coccidioidomycosis.

SOURCE: Figure courtesy of John N. Galgiani (data from the California Department of Health and Arizona Department of Health Services).

patients lost more than a month of work; a quarter of patients needed 10 or more physician visits; and 40 percent of patients required hospitalization for their illness (Tsang et al., 2010). The same report shows Arizona hospital costs for coccidioidomycosis were more than \$86 million. Extrapolating from these costs, estimates that include all outpatient medical care, often lasting for years if not entire lives, could easily reach a quarter of a billion dollars.

As significant as these findings are, other projections suggest that the actual number of persons seeking medical attention for coccidioidal infection is several times greater than those diagnosed and included in state public health statistics. One study found that only 3–13 percent of patients with pneumonia in Phoenix were tested for coccidioidomycosis (Chang et al., 2008). By contrast, a prospective study in Tucson in which patients with community-acquired pneumonia were tested for coccidioidomycosis demonstrated that nearly a third of these subjects had a coccidioidal infection (Valdivia et al., 2006).

State statistics show case rates for college-age persons in Pima County to be from 34 to 48 cases per 100,000 annually. However, recent surveillance of scholarship athletes at the University of Arizona, which is in Pima County, indicated 374 cases per 100,000 (Stern and Galgiani, 2010). Further analysis suggested that the most important reason for this much higher case rate was that the athletes received many more serologic tests for coccidioidomycosis. Evidently, more patients would be accurately identified as to the true cause of their illness if patients with endemic exposure to *Coccidioides* spp. were tested more routinely for this possibility. This is now the recommendation of the Arizona Department of Health Services and a growing number of Arizona state medical specialty societies and other professional organizations (Tsang et al., 2010).

With the commercial and recreational growth of the southwestern United States, coccidioidomycosis has become an increasing problem for the rest of the country as well. For example, persons who develop a respiratory illness within a month after returning from vacation or business conferences in south-central Arizona would have the same risk (approximately 30 percent) that their illness is due to *Coccidioides* spp. as would residents of the endemic regions. Using Arizona Department of Tourism statistics for 2008, the chance of an individual visitor developing any clinical illness would be expected to be small (approximately 1 in 17,000). However, because more than 22 million persons visit Arizona for an average of 4 to 5 days, the total number of illnesses occurring after leaving Arizona would add up to more than 1,300 per year. Evidence suggests that most of these illnesses would be diagnosed incorrectly in the course of routine medical care (Standaert et al., 1995).

Even if physicians obtain appropriate testing, establishing a diagnosis of early coccidioidal infection is often difficult. Coccidioidal serology is very specific when results are positive. Moreover, in progressive forms of infection, serology is very likely to be diagnostic (Fish et al., 1990; Pappagianis and Zimmer, 1990). However, these tests are not nearly as sensitive early in the course of the

acute pneumonia syndrome, the most common manifestation of a coccidioidal infection. In one study, depending on the method of analysis, false-negative results were estimated to occur from one third to two thirds of the time on first testing (Wieden et al., 1996). Although improved methods for detecting *Coccidioides*-specific antibodies may improve the sensitivity, approaches such as detection of coccidioidal DNA by polymerase chain reaction (Clark and McAllister, 1996) or detection of coccidioidal antigens by enzyme-linked immunosorbent assay (ELISA) (Durkin et al., 2008; Helfrich et al., 2011) offer theoretical advantages to earlier detection. Improvements in early diagnosis would be very useful to clinicians trying to manage their patients.

Therapy for Coccidioidomycosis and Its Current Limitations

The most serious complications of coccidioidomycosis include progressive chronic pneumonia and hematogenous spread of infection to parts of the body outside of the chest. Patients with these problems have benefited greatly from the advent of orally absorbed azole antifungal agents (Galgiani et al., 2005). Fluconazole and itraconazole are commonly used for these complications (Galgiani et al., 1993, 2000; Tucker et al., 1990). The more recently available azoles, voriconazole and posaconazole, offer additional options (Catanzaro et al., 2007; Prabhu et al., 2004; Proia and Tenorio, 2004; Stevens et al., 2007). This class of antifungal drugs has been found to be relatively safe and generally well tolerated for extended courses of administration. However, approximately a quarter of patients with such infections do not respond adequately to these drugs. Even in patients who appear to respond to azole treatment, relapses occur in approximately a third of those when treatment is discontinued. Thus, for some patients, including all patients with coccidioidal meningitis, treatment is recommended to be lifelong (Dewsnup et al., 1996). The most currently available drugs offer a suppressive effect; they do not eradicate infections or cure patients.

Surprisingly, there is virtually no published experience on the use of any antifungal drug for the most common manifestations of coccidioidal infection, that of the early respiratory syndrome. In a recent prospective observational study (Ampel et al., 2009), patients treated with oral azoles (usually fluconazole) appeared neither to improve at a faster rate nor subsequently to avoid progressive complication than did patients who did not receive antifungal treatment. Although not a randomized controlled trial, this study presents the only information available and provides little encouragement for the value of early treatment.

Prospects for new drugs to treat coccidioidomycosis are limited (Ostrosky-Zeichner et al., 2010). This is due in part to the general contraction in anti-infective drug development in general (Talbot et al., 2006), but is especially problematic for coccidioidomycosis because of its status as an orphan disease, defined by the Food and Drug Administration (FDA) as having a U.S. prevalence of less than 200,000. Even for new antifungals, such as voriconazole, posacon-

azole, and caspofungin (Gonzalez et al., 2001, 2007), which already have FDA approval for other fungal diseases, there are very limited or no controlled clinical trials conducted in patients with any form of coccidioidomycosis.

One exception to this pattern has been the persistent interest in bringing nikkomycin Z into clinical trials. Nikkomycin Z is a competitive inhibitor of chitin synthase, first discovered by German scientists in the 1970s. It was part of a fungicide discovery program at the Bayer Company (Fiedler, 1988). Its potential as a therapeutic for coccidioidomycosis was identified in the 1980s (Hector et al., 1990). In mice, nikkomycin Z treatment produced sterile lungs under conditions in which the lungs of untreated mice yielded several million viable fungal colonies. This observation raises the possibility that nikkomycin Z might offer a curative treatment for coccidioidomycosis. If so, this would provide even more incentive to diagnose coccidioidomycosis early in order to eradicate it and thereby prevent later and serious complications. Clinical development of nikkomycin Z was begun in the 1990s by Shaman Pharmaceuticals (Galgiani, 2007). However, the program became inactive when Shaman ceased to exist in 2000, and for several years nikkomycin Z development remained dormant.

In 2005, the University of Arizona acquired the program along with several kilograms of bulk nikkomycin Z that remained from Shaman's program. Since then faculty at the University of Arizona and a small start-up company, Valley Fever Solutions, have successfully competed for research awards and small business grants from the National Institutes of Health (NIH) and the FDA Office of Orphan Products Development. With these funds as well as philanthropic support, clinical trials with nikkomycin Z were resumed with a 2-week multidose safety trial that was completed in 2009. This support is also being used to develop a more efficient manufacturing process. Supplies of nikkomycin Z made by this new process are planned to be available to begin a Phase II clinical trial in 2011 or 2012. It is hoped that this progress will advance the program sufficiently to attract pharmaceutical or investment interest to complete the commercialization process.

Prospects for a Preventative Vaccine

A large majority of the estimated 150,000 U.S. coccidioidal infections occurring annually resolve with or without symptoms and whether or not specific antifungal treatment is instituted (Galgiani et al., 2005). Healing occurs as a result of the patient's cellular immunity, which is remarkably durable, usually lasting a lifetime (Galgiani et al., 2010). The fact that natural infection so often produces resistance to second infections has attracted a long-time interest in engendering this immunity through active immunization, leading to a clinical trial of a whole-cell killed vaccine (Pappagianis and Valley Fever Vaccine Study Group, 1993). This preparation did not produce protection. In retrospect it was surmised that the irritation at the injection site of the fungal cell wall polysaccharides prevented sufficient doses of protective immunogens to be delivered during vaccination. For

the past 15 years, a collaboration of several research groups has yielded a number of immunogenic coccidioidal proteins and vaccines prepared from recombinant proteins with adjuvants, some of which have shown excellent protection in mice and efficacy in primates (Cole et al., 2004; Cox and Magee, 2004; Herr et al., 2007; Johnson et al., 2007; Shubitz et al., 2006; Tarcha et al., 2006).

The next step for existing recombinant vaccine candidates would be for them to be moved into clinical trials. However, these candidates have met with major challenges including developing a suitable manufacturing process; identifying a suitable and available adjuvant; and compounding a suitable formulation appropriate for human experimentation (Galgiani, 2008). None of these challenges are insurmountable, but all require significant development investment. The overall impact of coccidioidomycosis within the endemic region is not so dissimilar to that caused by polio in the United States before a polio vaccine was available (approximately 10 per 100,000 population). However, the impact of coccidioidomycosis involves a much smaller population at risk as compared to the worldwide distribution of polio. This difference in market size makes it unlikely that a commercial vaccine manufacturer will invest in developing a coccidioidal vaccine even though such a vaccine, once developed, could arguably be profitable to manufacture and distribute (Barnato et al., 2001). Moving a coccidioidal vaccine into clinical trials probably requires the discovery of new, more easily formulated protective antigens; a breakthrough in vaccine technology that greatly reduces the cost of development; or a growing public health imperative to underwrite the costs needed for vaccine development.

Summary

Coccidioidomycosis is a major public health problem for a major, growing segment of the U.S. population as well as other endemic regions throughout the Western Hemisphere. A more complete understanding of its biology and ecology where it exists in the endemic environment could lead to risk abatement strategies not currently available. Improved recognition by healthcare professionals of coccidioidomycosis as a cause of community-acquired pneumonia when it occurs in their patients could improve management. This could be assisted further by developing more sensitive and clinically available diagnostic tests based on biosignatures such as DNA or proteins from the fungus itself. Curative therapies are also needed, but none exist today. Finally, eliminating problems caused by *Coccidioides* spp. might be possible if a preventive vaccine were developed. Even though coccidioidomycosis is an orphan disease, pursuit of these objectives is more than justified by the potential public health benefit and the reduced medical costs to society that their achievement would provide.

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Disclosure

Dr. Galgiani is chief medical officer, chair of the board, and a significant stock holder in Valley Fever Solutions, Inc.

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A9

CRYPTOCOCCUS GATTII: AN EMERGING PATHOGEN IN THE UNITED STATES

Julie R. Harris³⁰

The genus *Cryptococcus* comprises 37 different species, of which only 2 are relevant for clinical infection (*C. gattii* and *C. neoformans*). *Cryptococcus* spores are inhaled from the environment, causing a primary lung infection that may or may not be symptomatic. Disseminated disease may result in meningitis and death (Li and Mody, 2010). Intricately linked with severe immunosuppression, *C. neoformans* was rarely reported before the 1950s, when cancer treatments and organ transplants—conditions that often require immunosuppressive treatments—began occurring with increasing frequency (Perfect and Casadevall, 2011). The era of AIDS led to an exponential increase in the

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numbers of immunosuppressed persons, and a corresponding massive increase in *C. neoformans* infections worldwide (Mitchell and Perfect, 1995; Perfect and Casadevall, 2011). Today, cryptococcal infections due to *C. neoformans* are among the most common AIDS-defining infections (Park et al., 2009; Warkentien and Crum-Cianflone, 2010). In contrast, infections caused by *C. gattii* are reported much less frequently. Gatti and Eeckels produced the first report of *C. gattii* infection in 1970, from a 7-year-old boy in Congo in 1966 (Gatti and Eeckels, 1970). The boy was found to have a cryptococcal infection clinically similar to *C. neoformans*, but with a different pathogen morphology (Gatti and Eeckels, 1970). The newly observed pathogen was deemed to be a variant of *C. neoformans* called *C. neoformans* var. *gattii*.

Today, *C. gattii* is considered its own species (Kwong-Chung et al., 2002). During the mid-1980s, studies of geographic sources of *C. gattii* and *C. neoformans* isolates demonstrated that although *C. neoformans* was found from all areas of the world, *C. gattii* was found only in tropical and subtropical climatic zones (Kwon-Chung and Bennett, 1984a,b). The authors concluded that *C. gattii* was likely to be restricted to locations where the minimum winter temperatures typically remained above freezing (Kwon-Chung and Bennett, 1984a). During the next decade, limited information became available about the epidemiology of *C. gattii*, much of it from papers describing infections in endemic Australia and Papua New Guinea (Ellis, 1987; Mitchell et al., 1995; Seaton et al., 1996b, 1997; Speed and Dunt, 1995). These studies demonstrated that *C. gattii*, unlike *C. neoformans*, was found almost exclusively in immunocompetent persons. In agreement with the findings of Kwon-Chung and Bennett (1984a), *C. gattii* infections were still seen only in patients living in tropical and subtropical climates (Chen et al., 2000; Lalloo et al., 1994; Laurenson et al., 1993, 1996, 1997; Mitchell et al., 1995; Seaton et al., 1996a, 1997; Slobodniuk and Naraqi, 1980; Speed and Dunt, 1995).

Beginning in 1999, the rate of cryptococcal infections among HIV-uninfected persons living on temperate Vancouver Island, British Columbia (B.C.), Canada, began increasing rapidly (Hoang et al., 2004; Fyfe et al., 2002). Early investigations demonstrated that most of these infections were caused by *C. gattii*, rarely reported before from Canada (Kwon-Chung and Bennett, 1984a). Veterinarians, too, noted that a wide range of animals were becoming infected with *C. gattii* where they had not previously been found with non-*neoformans* cryptococcosis (Duncan et al., 2006). During the following years, the disease continued spreading in B.C., infecting humans and animals on the nearby mainland who had no history of travel to Vancouver Island (MacDougall et al., 2007), and by 2007, 218 human infections had been reported from B.C. Although four genetic groups of *C. gattii* have been identified (VGI, VGII, VGIII, and VGIV), most infections in British Columbia were caused by the relatively uncommon VGII genotype. Due to the high numbers of isolates available from the outbreak, further genetic subdivision of outbreak-associated isolates was performed, demonstrating that

approximately 90 percent of isolates in B.C. were of a “major strain” genetic subtype VGIIa, with a smaller number of “minor strain” subtype VGIIb isolates (Galanis and MacDougall, 2010).

Clinicians began noting *C. gattii* infections among patients in the Pacific Northwestern (PNW) states of Washington and Oregon in 2004 and 2005, respectively (MacDougall et al., 2007). In addition to the VGIIa and VGIIb infections, a new genotype of infection was noted in the United States, called VGIIc (Byrnes et al., 2010a). Beginning in October 2009, the Pacific Northwest *Cryptococcus gattii* Public Health Working Group, comprising the U.S. Centers for Disease Control and Prevention (CDC) and state health departments and laboratories in the Pacific Northwest, began retrospectively and prospectively collecting standardized information on *C. gattii* infections in the United States. As national awareness about the outbreak began to grow, other states also reported rare infections or submitted isolates for speciation and genotyping at the CDC.

Outbreak of *C. gattii* in the United States

To date, nearly 80 laboratory-confirmed human cases have been reported in the United States (Harris et al., 2010), most from Washington and Oregon (Figure A9-1). As awareness of the outbreak in the PNW has spread throughout the United States, infections from other states have also been reported. Case counts have increased each year, from a single case reported during 2004 to 24 cases reported during 2010 (Figure A9-2). The increase in reported cases is likely to be a result of both improved surveillance, as indicated by the recent increase in cases being reported from areas outside the PNW (Figure A9-2), and actual increases in case occurrences.

Among human patients in the United States identified through surveillance as having laboratory-confirmed *C. gattii* infection, approximately half are male, with a median age of 56 (range, 15–95). Patients aged 30 and older comprise the majority of cases (Table A9-1). The most commonly reported symptoms are headache, nausea, and cough, affecting more than half of all patients; more than half present with pneumonia and approximately half have meningitis. Most (73 percent) patients have an underlying immunosuppressive condition, including (but not limited to) a recent history of oral steroid use; lung, heart, kidney, or liver disease; a history of cancer; or a solid organ transplant (Table A9-2). HIV infection was more frequent among *C. gattii* patients than is found in the general U.S. population (5.9 percent vs. 0.6 percent) (CIA World Factbook, 2010), but was still the least commonly reported underlying condition. Of 59 patients with data, 17 (29 percent) had no detectable underlying immunocompromising condition. Cryptococcomas, or fungal masses, were found in the lungs and/or brains of substantial proportions of patients who received the corresponding scans or x-rays. Nearly all patients were hospitalized, and nearly one third with follow-up information died with or from their infections (Table A9-1).

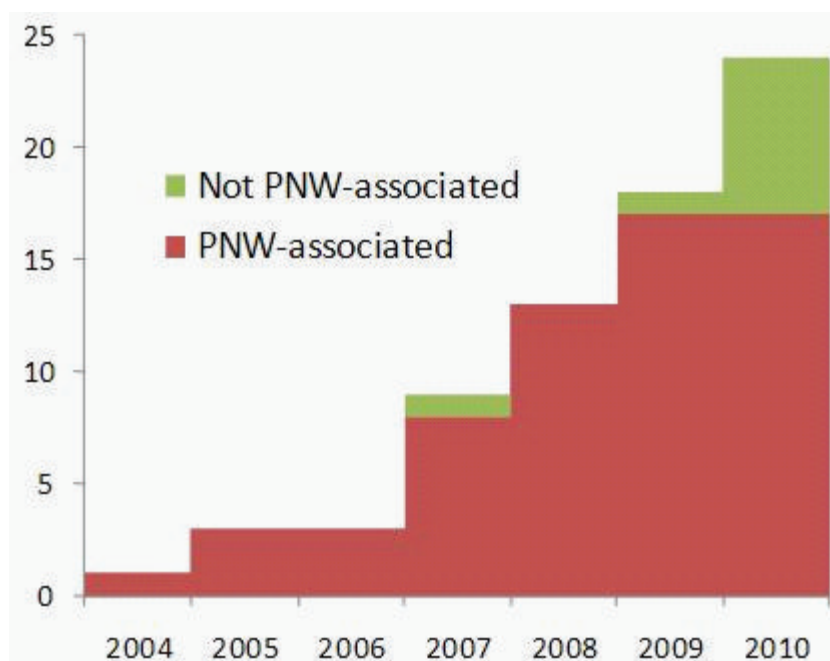


FIGURE A9-2 U.S. human cases of *C. gattii*, by year of illness onset (n = 71*).

* NOTE: Onset year is reported for 62 patients and is estimated by initial report year for 9 patients for whom onset date was not available. 2010 data are current as of January 2011; complete case data typically lag illness onset by several months.

PNW = Pacific Northwest.

While a small number of non-outbreak-strain infections have occurred in the PNW, most have been reported from states outside the PNW. A patient from North Carolina was diagnosed with *C. gattii* (genotype VGI) in 2007 following travel to San Francisco (Byrnes et al., 2009), and two cases of *C. gattii* (genotypes VGI and VGIII) were reported in 2010 from patients in New Mexico who did not have any recent history of travel outside of the state (Harris et al., 2010). In 2010, a patient was reported from Hawaii with a novel subtype of infection belonging to the genotype VGII (Harris et al., 2010). Since 2004, two patients have been re-reported from California and one from Michigan with VGIII-type infections. Thus, at least one focus of infection involving genotypes novel in the United States appears to be ongoing in the PNW; it also appears that sporadic *C. gattii* infection is occurring elsewhere, with genotypes distinct from those found in the PNW.

The collection of standardized data on U.S. outbreak-strain and non-outbreak-strain infections has provided an opportunity to examine clinical

TABLE A9-1 Characteristics of *C. gattii* Patients in the United States, 2004–2010

Characteristic	n	N	%
Age (mean, median, range), years	52, 55 (15–95)		
≤ 18	3	64	5
19–29	3	64	5
30–49	23	64	36
50–69	27	64	42
70+	8	64	13
Symptoms			
Headache	32	55	58
Nausea	26	50	52
Cough	29	57	51
Dyspnea	24	51	47
Fever	25	57	44
Vomiting	22	50	44
Fatigue	17	40	43
Weight loss	21	50	42
Loss of appetite	15	46	33
Chills	16	52	31
Muscle pain	15	52	29
Chest pain	14	52	27
Neck stiffness	11	49	22
Night sweats	6	44	14
Photophobia	5	49	10
Seizure	4	45	9
Blurry vision	2	45	4
Any respiratory	39	55	71
Any central nervous system	19	44	43
Clinical findings			
Pneumonia	27	49	55
Meningitis	26	51	51
Crypto lung	18	50	36
Crypto head	6	22	27
Outcomes of infection			
Hospitalized	52	57	91
Died	16	52	31

differences by genotype and patient type. Although a relatively small number of non-outbreak-strain infections have been reported, significant differences between these and outbreak-strain infections have been noted in the proportion of infected patients who were immunocompromised and the propensity for respiratory and CNS symptoms (Table A9-2). It seems likely that different genotypes of *C. gattii* might infect different patient types; in addition, clinical manifestation of *C. gattii* infection might differ either by infecting strain genotype, patient immune status, or both.

TABLE A9-2 Comparison Between Outbreak-Strain (VGIIa/b/c) and Other Genotypes of Infection with *C. gattii*, United States, 2004–2010

Symptoms, Patient Characteristics, Outcomes	VGIIa/b/c	Other genotypes	RR	p
Respiratory symptoms				
Cough	58%	11%	5.3	0.01
Dyspnea	53%	12%	4.3	0.05
Any respiratory symptom	76%	44%	1.7	0.10
Central nervous system symptoms				
Seizure	3%	50%	0.05	0.01
Blurry vision	8%	43%	0.19	0.04
Any central nervous system symptom	34%	100%	0.04	0.00
Preexisting medical condition	78%	33%	7.1	0.01
Hospitalized	90%	100%	0.9	1.00
Died from or with <i>C. gattii</i>	32%	16%	2	0.65

NOTES: p: Fisher's exact p-value; RR: Relative risk

Historical Isolates of C. gattii in the United States

In spite of extensive press coverage in the United States during 2010 about the “new deadly fungus” in the United States and British Columbia (*Discover Magazine*, 2010; Hutchison, 2010; Park, 2010), *C. gattii* infections are not entirely novel in the United States: in particular, the infection repeatedly has been found in California in the past. In a 1984 study, *C. gattii* was found in 46 of 315 clinical isolates of *Cryptococcus* from the United States; 30 (65 percent) of the 46 *C. gattii* isolates were from patients residing in Southern California. Among all 71 *Cryptococcus* isolates from Southern California, *C. gattii* represented 42 percent, compared with 6 percent in the rest of the continental United States (Kwon-Chung and Bennett, 1984b). The same group reported in a different paper that both of the two clinical *Cryptococcus* isolates from Hawaii were *C. gattii* (Kwon-Chung and Bennett, 1984a). Another study, which examined cryptococcal isolates from HIV-infected patients from Los Angeles in the early 1990s, found *C. gattii* in 12 percent of these isolates (Chaturvedi et al., 2005). Genotyping of the isolates showed that 28 of 30 isolates were of subtype VGIII; 1 was VGI, and 1 was of subtype VGII (Byrnes et al., 2010b).³¹ *C. gattii* was also found in three of 358 genotyped isolates collected from around the United States during surveillance from 1992 to 1994; two isolates were from San Francisco (VGI and VGIII) and one was from Atlanta (VGI) (Brandt et al., 1996). Finally, two isolates of genotype VGIIa, indistinguishable by multilocus sequence typing (MLST) from the major strain in Vancouver Island, were collected from the sputum of a Seattle man in the 1970s (Diaz et al., 2000) and from a eucalyptus tree in San

³¹ Also see contributed manuscript by Heitman in Appendix A (pages 226–248).

Francisco in 1992 (Fraser et al., 2005). Taken together, these data suggest that Southern California, but probably not the rest of the country, has long been an endemic area for *C. gattii*. In addition, sporadic infections appear to be occurring from other states, although the travel history of these patients and their potential exposure site is unknown. Recently, environmental isolates of *C. gattii* have also been found in Puerto Rico from a variety of cacti and tree material (Loperena-Alvarez et al., 2010).

C. gattii in the PNW vs. *C. gattii* in Other Areas of the World

Globally, the most common infecting strains of *C. gattii* and the immune status of patients infected appear to vary. In endemic Australia and Papua New Guinea, *C. gattii* (usually type VGI) appears to occur primarily in apparently immunocompetent patients and cause CNS disease (Seaton et al., 1996a,b; Speed and Dunt, 1995). However, in several African countries, VGIV-type infections are most frequent, and are reported exclusively from HIV-infected patients (Litvintseva et al., 2005; Steele et al., 2010). By contrast, in Venezuela (Villanueva et al., 1989), Brazil (Santos et al., 2008), Paraguay, Argentina (Castanon-Olivares et al., 2000; Kwon-Chung and Bennett, 1984a,b), Peru (Bustamante Rufino and Swinne, 1998; Kwon-Chung and Bennett, 1984a,b), and Colombia (Escandon et al., 2006), infections (most commonly genotype VGII, although different from the PNW outbreak strains) occur in both HIV-uninfected and HIV-infected patients. In Mexico, all four *C. gattii* genotypes have been reported (Olivares et al., 2009), primarily from HIV-uninfected persons (Castanon-Olivares et al., 2000; Lopez-Martinez et al., 1996). In Asia, *C. gattii* (primarily VGI and VGII) has been reported from Vietnam, Cambodia, Thailand, Korea, Japan, Malaysia, India, China, and Nepal (Chen et al., 2008; Choi et al., 2010; Kwon-Chung and Bennett, 1984a,b; Lui et al., 2006; Okamoto et al., 2010), in both immunocompetent and immunocompromised persons (Chau et al., 2010; Ngamskulrungroj et al., 2010).

The outbreak of *C. gattii* in B.C. and the PNW is qualitatively different in genotype, presentation, disease course, and outcome than has been seen with *C. gattii* in other locations. In contrast to findings from areas outside of North America, *C. gattii* patients in both B.C. and the PNW most commonly present with respiratory rather than CNS symptoms (Galanis and MacDougall, 2010; Harris et al., 2010). In addition, a high proportion of infections in both the PNW and B.C. occur in immunocompromised (but usually HIV-uninfected) patients. In B.C., 38 percent of patients are immunocompromised (Galanis and MacDougall, 2010); using the same case definition for immunocompromised state, in the United States, approximately 59 percent of patients are immunocompromised (CDC, unpublished data).

Interestingly, differences also might exist between *C. gattii* patients in the United States and in B.C. U.S. patients were younger, more likely to be hospital-

ized, and more likely to die from or with their infection than were B.C. patients (Harris et al., 2010). The reasons for these differences are unclear, but might include differences in case ascertainment in the PNW compared with B.C., which has led to capture of a lower proportion of non-hospitalized *C. gattii* patients in the PNW. Alternately, they could relate to the different genotypes seen in the PNW compared with B.C. A comprehensive review of patient medical charts is under way. It might help elucidate the differences and provide some insight into whether these differences are real, and if so, why they exist.

The differences in U.S. *C. gattii* infections in the PNW, U.S. infections outside the PNW, and infections occurring in other areas of the world might be a function of *C. gattii* subtype, tropism, environmental distribution, or surveillance bias. That is, *C. gattii* infections might not be reported completely from other areas because they have not been looked for systematically elsewhere. Regardless of the reasons for the outbreak in North America, reports of the outbreaks do not appear to be exclusively due to temporal changes in surveillance. Retrospective speciation studies of isolates from B.C. before 1999 (Fyfe et al., 2008) and from the Seattle area before 2004 (Upton et al., 2007) suggested that the increase in reported cases represents a true increase in infections in the region. It remains to be seen whether the U.S. *C. gattii* infections outside of the PNW are sporadic, travel associated, or linked to part of a larger emerging health issue in other areas of the United States. Below is a discussion about efforts to conduct surveillance for *C. gattii* infections outside of the PNW.

Surveillance for Human C. gattii Infections Outside the PNW

To address the question of how frequently *C. gattii* infections are occurring outside of the PNW, in October 2010 the CDC issued an alert through ClinMicroNet (Dwyer, 2003), a listserv sent to directors of U.S. clinical microbiology laboratories. The alert described the ongoing outbreak in the Pacific Northwest, and requested that any unspciated *Cryptococcus* isolates (or those already speciated as *C. gattii*) be sent to the Mycotic Diseases Branch laboratory at the CDC, with isolation date, source city, and source state. Isolates were speciated at CDC and *C. gattii* isolates were genotyped. As of January 2011, 32 isolates, isolated between 2006 and 2010, from patients in seven states had been submitted to the CDC. Of these, 10 were *C. gattii*; half were from California (Table A9-3). Collection of isolates is ongoing and cases continue to be reported to the CDC, albeit rarely, from states outside of Washington and Oregon. The recent commercial availability of specialized culture medium (CGB medium) that enables rapid discrimination between *C. neoformans* and *C. gattii* will hopefully facilitate this process (Butler-Wu and Limaye, 2011).

TABLE A9-3 Sources and Species of Isolates of *Cryptococcus* Submitted Following a Request Through ClinMicroNet, United States, October 2010–February 2011

State	<i>C. neoformans</i> isolates	<i>C. gattii</i> isolates	Total isolates
AL	3	0	3
CA	1	5	6
GA	1	2	3
HI	0	1	1
IL	14	0	14
OR	0	1	1
TX	3	0	3
UT	0	1	1
Total	22	10	32

Clinical Aspects of *C. gattii* Infection and Differences from *C. neoformans*

Several attempts have been made to carry out head-to-head comparisons of clinical disease caused by *C. gattii* and *C. neoformans*. In 1995, Speed and Dunt (1995) published a paper comparing clinical symptoms among hospitalized *C. gattii* and *C. neoformans* patients. In addition to noting that fewer than 10 percent of *C. neoformans* infections in Australia occurred in otherwise healthy patients while 100 percent of *C. gattii* infections occurred in healthy patients, the authors also reported that *C. gattii* more frequently involved cerebral and meningeal sites, had neurologic sequelae, required CNS or thoracic surgery to resect cryptococcomas, and required longer periods of treatment (Speed and Dunt, 1995). In addition, *C. gattii* infections more frequently resulted in relapse than *C. neoformans* infections. The authors commented that the nearly three-fold longer therapy times required for patients with *C. gattii* compared with patients with *C. neoformans* infections stemmed from the difficulty in reducing the size of the cryptococcomas and the inability to rapidly control infection in patients with *C. gattii*. However, they also noted that both bloodstream infections and mortality were exclusively limited to patients with *C. neoformans* (Speed and Dunt, 1995).

The same year, Mitchell et al. (1995) published a report directly comparing the two cryptococcal species in immunocompetent patients with cerebral disease (Mitchell et al., 1995). Similar to the findings by Speed and Dunt (1995) and later by Chen et al. (2000), the authors reported that both lung and brain cryptococcomas were more common among patients with *C. gattii* than *C. neoformans* infections. In addition, they reported that immunocompetent patients with *C. gattii* infection were significantly more likely to have a “poor outcome”—defined as moderate to major sequelae or death—than immunocompetent patients with

C. neoformans infections. When they compared outcomes among patients with meningitis but normal brain imaging at initial presentation, they found no differences by infecting species; however, they did find that *C. gattii* patients presenting with mass lesions on initial intracranial scan were more likely to have poor outcomes than those who did not (Mitchell et al., 1995). Taken together, these data suggested that the epidemiology of *C. neoformans* and *C. gattii* in Australia was different even when controlling for patient immune status.

However, a similar paper, published in 2010, compared infection with *C. gattii* and *C. neoformans* in immunocompetent patients in Vietnam (Chau et al., 2010) and failed to demonstrate differences in clinical phenotype by infecting species. The authors of this report, in contrast to Mitchell et al. (1995), suggested that host immune status was more influential on clinical course and outcome than was infecting species. This was also a conclusion drawn by Chen et al. (2000), who compared cryptococcal infections among both immunocompetent and immunosuppressed patients in Australia and New Zealand and demonstrated that immunocompetent hosts were more likely to present with lung infections, species type notwithstanding, than were immunocompromised hosts. Chen et al. (2000) also noted that *C. gattii* infections were more likely to occur in the brain than *C. neoformans* infections, but that cryptococcomas were associated both with infection with *C. gattii* and with immunocompetent status.

Lui et al. (2006) also compared cryptococcal infection in immunocompetent and immunosuppressed patients in China, noting elevated proportions of immunocompetent patients presenting with meningitis compared with immunocompromised patients, more intense inflammatory responses, and a lower risk of death. Although the paper also indicated that immune status might influence clinical course more than infecting species, the small numbers of *C. gattii* infections made evaluation of the effect of infecting species difficult.

Only two studies have directly compared *C. gattii* infections to *C. neoformans* infections exclusively in AIDS patients. In Botswana, Steele et al. (2010) compared *C. gattii* and *C. neoformans* infections in AIDS patients with cryptococcal meningitis, and found few differences in terms of clinical presentation or in-hospital mortality (Steele et al., 2010). Morgan et al. (2006) found similar results among South African patients with cryptococcal meningitis. It is possible that, among severely immunosuppressed patients who have a low capacity to respond immunologically to infection, such as late-stage AIDS patients, the infecting cryptococcal species is irrelevant to outcomes, while infecting species have a stronger influence when patients are mildly to moderately immunosuppressed or not immunosuppressed. These studies did not carry out extensive brain or thoracic imaging to evaluate the presence of cryptococcomas.

The poor prognosis of immunocompetent patients infected with *C. neoformans* has been studied previously, and has been suggested to be due to the delay in diagnosis, inappropriate treatment, and potentially the presence of an intact immune system that might provoke an immune reconstitution inflammatory

syndrome (IRIS) (Ecevit et al., 2006; Lui et al., 2006). IRIS is a paradoxical clinical deterioration that is well documented during treatment of cryptococcosis following initiation of antiretroviral therapy in AIDS patients (Woods et al., 1998) and is thought to be due to an overzealous “rebound” immune response in the presence of significant amounts of infecting pathogen. An IRIS-like syndrome has also been documented in patients infected with *C. gattii*, where the syndrome was suggested to be due to concomitant immune rebound and decreases in IL-10 (Einsiedel et al., 2004). These same factors may contribute to the severity of *C. gattii* infection in immunocompetent hosts. At least one report exists of a patient whose condition improved with steroid treatment, suggesting that an overly functional immune system could confound treatment efforts in some patients (Lane et al., 2004).

Discussion

The described outbreaks of *C. gattii* infection in the temperate climates of B.C. and the PNW demonstrate a much less restrictive geographic range for *C. gattii* than previously thought, and a broader range of persons who are susceptible to infection. In particular, a compromised immune status now appears to be a significant risk factor for at least some subtypes of *C. gattii* infection (CDC, 2010; Galanis and MacDougall, 2010). In addition, data from patients associated with these outbreaks suggest that different *C. gattii* genotypes might infect different types of patients, and/or demonstrate different clinical courses resulting from infection. The mere existence of an outbreak associated with *C. gattii*, never previously reported, suggests that genetic components might be important for pathogen spread in ways that are still poorly understood. More than ever, collecting data is important that disease recognition and optimal treatment of *C. gattii* infections can be investigated. Several existing challenges now face the field.

Existing Challenges

One existing challenge is diagnosis of infection. Although several methods exist to identify cryptococcal infections, including culture, India Ink stains of cerebrospinal fluid or sputum (Cohen, 1984), and commercially available cryptococcal antigen (CrAg) test kits (Saha et al., 2008), these methods cannot distinguish between *C. neoformans* and *C. gattii*. A simple way to confirm whether or not a cryptococcal isolate is species *gattii* is to plate the isolate on canavanine-glycine bromothymol blue (CGB) agar (Klein et al., 2009; Kwon-Chung et al., 1982), where *C. neoformans* will leave the medium unaffected in color (yellow to green) due to a failure to grow, and *C. gattii* will turn the medium blue due to use of glycine as a carbon source. This medium is currently available from at least one commercial supplier, but is not widely used in U.S. clinical microbiology labs. Thus, many *C. gattii* infections likely are being misdiagnosed as

C. neoformans. Ensuring that clinicians are aware of *C. gattii* infection and the possible need for clinical differentiation from *C. neoformans*, and that their reference laboratories are able to speciate *Cryptococcus* isolates (and have an interest in doing so), is critical to evaluate fully the geographic spread of disease and the clinical spectrum of infections.

Investigating whether or not the most recent findings warrant modified treatment guidelines is an additional challenge. The Infectious Diseases Society of America published guidelines in 2010 (Perfect et al., 2010) that refer to differences in the treatment of *C. gattii* infections, compared with *C. neoformans* infections: specifically, *C. gattii* infections might require lengthier, more aggressive treatment when compared with *C. neoformans* infections. The increased propensity for *C. gattii* to form cryptococcomas is also noted (Perfect et al., 2010). However, the guidelines were largely based on data from *C. gattii* infections occurring in Australia and Papua New Guinea. Increasingly, our data suggest that even among *C. gattii* infections, not all cryptococcal infections are alike. However, it is unclear which factors—infesting species, infesting subtype, host immune status, or perhaps even host genetics—are most influential on patient presentation and infection. Data from rigorous clinical studies are of utmost importance in ensuring that clinician guidelines provide sufficient guidance to optimize patient care. To this end, a large-scale, longitudinal chart review of *C. gattii* infections is ongoing as a collaborative effort among Australia, B.C., and the United States, designed to address some of these questions. Results are expected sometime in 2012.

Finally, the development of prevention messages is a challenge. Unlike *C. neoformans*, which grows in pigeon feces, *C. gattii* appears to live in association with trees and soil surrounding them (Springer and Chaturvedi, 2010). The tree type appears to be less important than the presence of a wood substrate for growth, and *C. gattii* has to date been associated with more than 50 tree species (Randhawa et al., 2001; Springer and Chaturvedi, 2010). It has also been found in air and water samples. These findings notwithstanding, *C. gattii* has not been found ubiquitously around the globe in a distribution similar to *C. neoformans*, and thus we can postulate that at least some environmental restrictions remain in place for this organism. Environmental organisms present a specific challenge for public health prevention because infections are usually relatively rare, and difficult to avoid without draconian measures (e.g., staying indoors and purifying air). This represents a quandary for public health officials. It remains to be seen whether “hot spots” of infection exist in the PNW for which generalized recommendations can be made that would benefit patient health, perhaps for subgroups of higher risk patients. The benefits of outdoor activity would need to be weighed against any risk calculated for these patients, and such recommendations are bound to be controversial.

Where Did C. gattii Come From, and Where Is It Going?

How did *C. gattii* arrive in the temperate areas of North America? Kidd et al. (2007) demonstrated, during an environmental sampling study for *C. gattii* in B.C., that anthropogenic activities could spread the pathogen, showing the presence of *C. gattii* on the shoes of human samplers and wheel wells of sampling vehicles as they traveled from one sampling site to the next. In addition, they showed that the pathogen was present in highly trafficked areas of Vancouver Island; that it could be found in the air, in freshwater, and in seawater around the area; and that the spores could survive for more than a year in many of these media. Thus, it is not difficult to hypothesize several methods by which the pathogen could have found itself in temperate B.C., and easier still to imagine mechanisms by which it could be transported into the United States. However, the question of whether new, cold-tolerant strains of *C. gattii* were brought to B.C. or whether they were formed there, through recombination of two or more preexisting or “seeded” strains, is still unresolved. It is also possible that the pathogen was always tolerant of temperate-weather climates, but existed in caches too small in these regions to sustain human infection or to establish permanent habitats; repeated seeding of these regions through global materials trafficking (e.g., wood or trees) could have created a sustainable niche for the pathogen. Alternately, changes in the global climate could be facilitating the optimal habitat development and spread of *C. gattii*, providing minimum conditions under which the pathogen can successfully propagate. Whole-genome sequencing is currently being carried out with *C. gattii* isolates obtained from patients associated with this outbreak, as well as more historical isolates (Lockhart et al., 2010), which could shed some light on the origin of the current outbreak and provide ideas for where it might move next.

Conclusion

The increase in the number of reports during the past decade related to the occurrence of *C. gattii* infections outside of traditional endemic tropical and subtropical regions has provided excellent opportunities to learn more about this important pathogen. The differences between individual cryptococcal infections appear to be linked not only to patient immune status and infecting species, but also to genetic subtypes within a species. It is unclear if the species and subtypes have preferences for infection among certain patient types, possibly due to a need for host immune support (or lack thereof) for replication, or if differences in environmental colonization patterns might influence the type of patient infected. For example, a pathogen with a ubiquitous distribution and a preference for immunocompromised patients will have a much higher infection rate *and* a much higher immunocompromised to immunocompetent patient ratio than would a pathogen with “hot spot distribution,” which would infect fewer patients overall, but be limited to patients living in its area of environmental distribution (most of

whom are immunocompetent). Thus, the immunocompromised to immunocompetent ratio might be nothing more than a function of the degree of distribution of a *Cryptococcus* species in the environment and the types of patients living in its area of distribution. Determining what governs the range of distribution of *C. gattii*—and understanding when it reaches a stable environmental equilibrium in new areas of emergence—is critical for understanding this relationship.

In spite of the recent flood of reports about *C. gattii*, much remains unknown. The epidemiologic curve has not yet stabilized in the United States, and the trajectory of future infections is unknown. The lack of comprehensive surveillance, both within North America and without, and the genetic variety inherent in *C. gattii*, has limited our current understanding of pathogen spread and pathogenesis. The conditions that favor pathogen colonization and propagation are not known. Evidence shows that infections in the United States and particularly the PNW are qualitatively different from those occurring elsewhere, but it is unclear whether or not these differences warrant modifications to existing treatment guidelines. Continued collection of robust surveillance data will assist in answering some of these questions. The coming years should see increasing amounts of information on *C. gattii* infections globally, which should shed light on genotype- and subtype-specific differences among *C. gattii* infections.

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A10

**SEXUAL REPRODUCTION, EVOLUTION, AND
ADAPTATION OF *CRYPTOCOCCUS GATTII* IN
THE PACIFIC NORTHWEST OUTBREAK**

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Abstract

How microbial pathogens emerge to cause outbreaks and become established as agents of disease in humans involves genetic exchange, zoonotic transmission, and perturbations of ecosystems and habitats. The threat of emerging infectious diseases is particularly poignant for eukaryotic pathogens, the fungi, and parasites, given that these microbes are more difficult to treat and have complex genomes and lifecycles. A sobering recent development has been the emergence and reemergence of several fungal pathogens in both humans and other animals, including *Geomyces destructans* in bats, *Batrachochytrium dendrobatidis* in amphibians, *Nosema ceranae* in bees (colony collapse disorder), and *Cryptococcus gattii* in humans and other animals in the Pacific Northwest. Here we review issues surrounding the *C. gattii* outbreak that began on Vancouver Island in 1999 and has expanded into the United States in Washington, Oregon, and California and has the potential to expand further. The focus will be on the emergence of *C. gattii* in the United States, including the appearance of a novel, highly virulent genotype and the potential role of sexual reproduction in the emergence of novel pathogens and their dispersal via airborne spores.

Introduction

The early history of cryptococcosis was documented in single or small series of cases. From an initial case of tibial osteomyelitis with the encapsulated *Cryptococcus neoformans* yeast in 1895 until a seminal monograph on this disease by Littman and Zimmer in 1956, the entire repertoire of reports in the medical literature numbered less than 300 cases (Littman and Zimmer, 1956). This was a humble beginning for this cosmopolitan, encapsulated basidiomycete that has now emerged into an outbreak mode in the new millennium. In the first half-century of its known existence, many of the clinical features of cryptococcosis were well described, including its propensity to invade the central nervous

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system. The occurrence of outbreaks of mastitis (persistent inflammation of the udders) in dairy herds in which hundreds of animals were infected brought the realization that cryptococcal infection outbreaks can occur in mammals (Pounden et al., 1952; Simon et al., 1953). These outbreaks in animals and the link to the environment again were emphasized with reports of goats developing *Cryptococcus gattii* infection in Spain temporally linked to the importation of Eucalyptus trees (Baro et al., 1998). These two outbreaks in animals vividly demonstrated that cryptococcal infections could change from sporadic to outbreak as the endemic status changes. However, the first prescient report that recognized the future emergence of human cryptococcosis was from Kaufman and Blumer at the Centers for Disease Control and Prevention (CDC) when they called cryptococcosis “an awakening giant” mycoses (Kaufman and Blumer, 1978). As clinical mycologists in a major reference laboratory, they observed increasing numbers of cases as the immunosuppressed population dramatically increased due to the use of immunosuppressive therapies in modern medicine. In 1983 to 1984, reports of opportunistic cryptococcal infections coinciding with the early natural history of HIV infection provided insights into the emerging association of HIV infection and cryptococcosis (Lerner and Tapper, 1984; Vieira et al., 1983). In a landmark epidemiologic work in 2009, Park and colleagues from the CDC estimated that, in association with the AIDS pandemic, there were approximately 1 million cases of cryptococcosis per year worldwide, with at least 600,000 deaths per year in the past 5 years due to cryptococcosis (Park et al., 2009). While this massive outbreak continues, on another front, an outbreak of *C. gattii* infections on Vancouver Island has been observed over the past decade and has now migrated down into the Northwest United States, infecting humans and other mammals (Byrnes and Heitman, 2009; Byrnes et al., 2009, 2010; DeBess et al., 2010; Fraser et al., 2005; Kidd et al., 2004; MacDougall et al., 2007; Upton et al., 2007).³⁵ Cryptococcosis started as a medical curiosity in a few patients, but because of medical interventions with immunosuppression, an immunosuppressive viral pandemic (HIV/AIDS), and a change in local climates, this yeast has become more prevalent in clinical medicine. Its present impact supports its title as a major emerging fungal disease or, to be more direct, “the giant is fully awake.”

Vancouver Island *C. gattii* Outbreak and Expansion into the United States

Our focus in this chapter will be the *C. gattii* outbreak that began on Vancouver Island in 1999 and has now expanded into the Canadian mainland in British Columbia and into the United States. A considerable body of knowledge is available with respect to the life and virulence cycles for this pathogenic yeast (Heitman, 2011). *C. neoformans* and *C. gattii* are closely aligned species. *C. neoformans* is prevalent in the environment globally, and *C. gattii* has been

³⁵ See also the contributed manuscript by Harris in Appendix A (pages 207–225).

thought to be geographically restricted to tropical and subtropical regions until its recent emergence in the relatively temperate climate on Vancouver Island. We are exposed to both organisms from the environment; *C. neoformans* is typically associated with pigeon guano and less commonly with trees, whereas *C. gattii* is commonly isolated from trees and soil, and also present in the air on Vancouver Island. We are exposed by inhaling spores and desiccated yeast cells, both of which can cause an initial pulmonary infection. This can be cleared, recede into a dormant latent form, cause fulminant pneumonia, or even make its way to the central nervous system via the bloodstream to infect both the covering of the brain (meninges) and the brain itself (meningoencephalitis). For reviews of the virulence and lifecycles, see Idnurm et al. (2005) and Kronstad et al. (2011).

Of particular importance are recent studies from the CDC's Park and colleagues (2009) that reveal that *Cryptococcus* has reached pandemic proportions. More than a million cases occur globally each year. This is largely in the context of the AIDS pandemic and results in more than 600,000 attributable mortalities, approximately a third of all AIDS-associated deaths. This is thought to be largely attributable to *C. neoformans*, but there is also likely to be a more substantial burden of *C. gattii* infection occurring globally than is currently appreciated given that few clinical microbiology laboratories routinely assign *Cryptococcus* species status. It is in this global context that we consider the expanding and ongoing outbreak of *C. gattii* in the Pacific Northwest (Figure A10-1).

Cryptococcus is a species complex, and it is important to know which pathogen you are dealing with in the context of infection. For example, this is particularly important with *E. coli*, in which various strains are causing infections such as EPEC, EHEC, UPEC, and VTEC (enteropathogenic *E. coli*, enterohaemorrhagic *E. coli*, uropathogenic *E. coli*, and verotoxin-producing *E. coli*). This is also true with fungal pathogens. Two species are currently recognized: *C. neoformans* and *C. gattii* (Figure A10-2). The two can be readily distinguished in clinical microbiology labs on L-canavanine glycine bromothymol blue (CGB) agar, exploiting the ability of *C. gattii* to cause a pH change on this media, resulting in a blue chromogenic reaction (Figures A10-2A and B). We now appreciate that the group of isolates currently recognized as *C. gattii* spans four cryptic species groups. Currently, these are recognized as the VGI, VGII, VGIII, and VGIV molecular types (Figure A10-2C) based on molecular phylogenetic analyses that show each as a distinct, well-defined group (Bovers et al., 2008; Fraser et al., 2005; Ngamskulrungronj et al., 2009a). We also know from the whole-genome analysis for the representative VGI (WM276) and VGII (R265) isolates, which has just been completed at the Broad Institute and the University of British Columbia in Vancouver by Jim Kronstad and colleagues, that at a whole-genome level the different molecular types are not exchanging genetic information (D'Souza et al., 2011). Similarly, the molecular types are four genetically isolated cryptic species based on robust analysis of "molecular barcodes" throughout their genomes us-

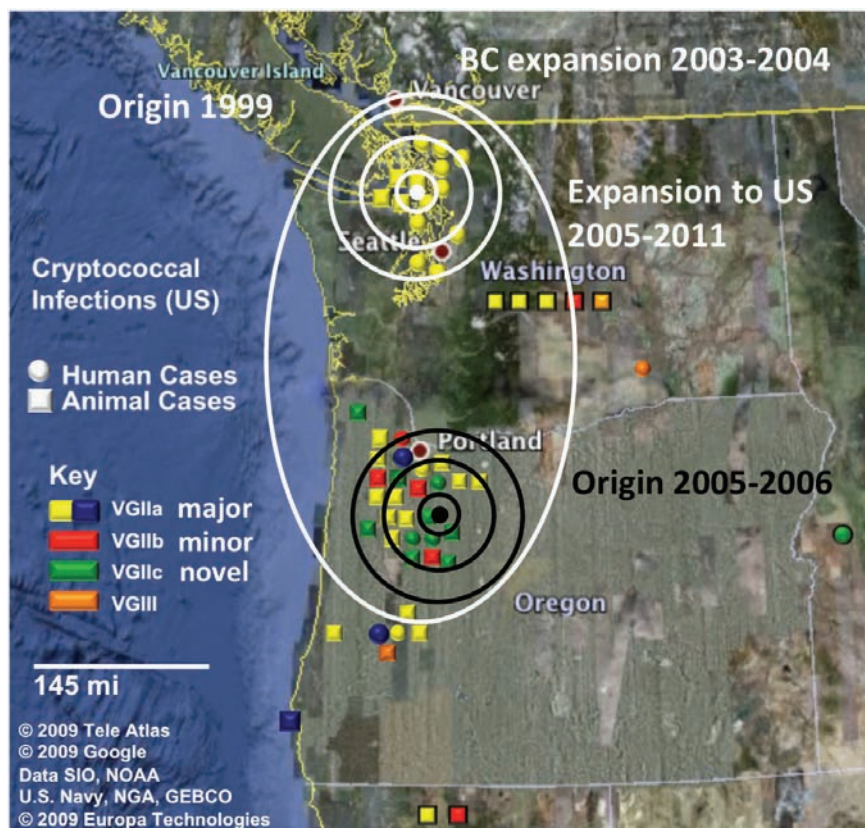


FIGURE A10-1 The *C. gattii* outbreak expanded into, and emerged within, the United States ($n = 56$). BC = British Columbia.

ing multilocus sequence typing (MLST) (Bovers et al., 2008; Fraser et al., 2005). This distinction of cryptic species is important because the VGII cryptic species is causing the outbreak in the Pacific Northwest, whereas the VGI lineage is more prevalent and more commonly causing disease in Australia (see Heitman, 2011).

The outbreak of *C. gattii* in the Pacific Northwest began in 1999 and prior to this, no *C. gattii* infections were reported as causing disease in this region of the world. The first cases came to the attention of astute clinicians and veterinarians on the southeastern shores in Nanaimo and Parksville on Vancouver Island (Duncan et al., 2005; Hoang et al., 2004; Stephen et al., 2002). Over the past decade, there have been approximately 260 cases with an approximately 10 percent attributable mortality rate, and many infections in animals. Interested read-

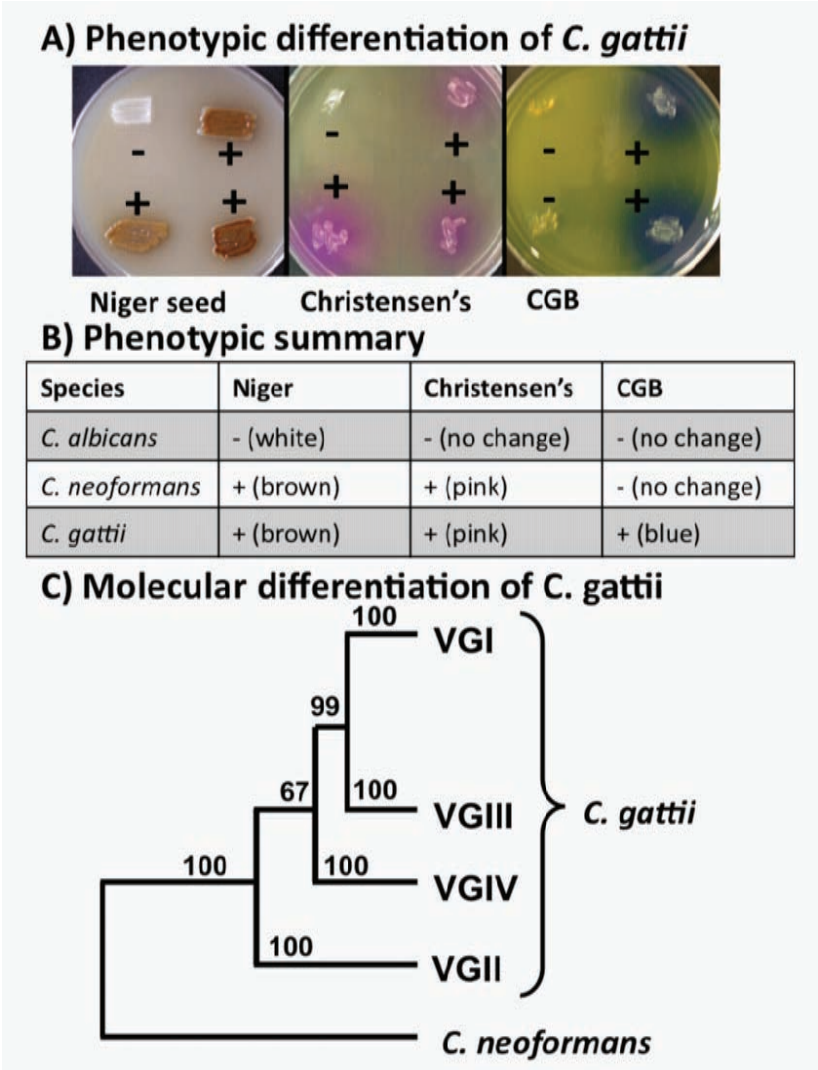


FIGURE A10-2 *Cryptococcus* pathogenic species complex. Panel A shows growth on niger seed media that detects production of the pigment melanin (+) or its absence (–), production of urease (+) as a pink color on Christensen’s agar, and growth and a blue color change (+) on CGB agar. Panel B shows a summary of the phenotypic data shown in panel A. Panel C is a phylogenetic tree of the relationships between the molecular types in the species complex. *C. neoformans*: opportunistic, AIDS, global, pigeon guano. *C. gattii*: primary, non-AIDS, tropical, arboreal. CGB = L-canavanine-glycine-bromothymol blue. SOURCE: Panel A modified from Byrnes et al., *PLoS Pathogens*, 2010 (figure S2). Panel B courtesy of Heitman. Panel C modified from Chapter 22, Figure 2 of Carter et al. (2011).

ers are referred to a reprint³⁶ included in this volume from Karen Bartlett, who played a critical role in identifying the environmental source of the organism on Vancouver Island (Bartlett et al., 2008), and to the manuscript from Julie Harris from the CDC *Cryptococcus* working group.³⁷ Our charge in this article was to consider two aspects of the outbreak: first, its expansion into the United States, and second, the possible roles of sexual reproduction in the origin of the outbreak isolates and the ongoing production of airborne infectious spores.

The first cases associated with the Vancouver Island outbreak in patients from mainland British Columbia with no travel history to the island appeared in 2003–2004 (Bartlett et al., 2008). Environmental sampling studies provided evidence that *C. gattii* expanded across the water to a broader niche, including the Canadian mainland (MacDougall et al., 2007). The southern tip of Vancouver Island is very close to the U.S. border, and therefore a key question was if and when the outbreak might spread into the United States. The San Juan Archipelago is a part of Washington state, located as near as 5 km from the gulf islands off the coast of Vancouver Island. The first *C. gattii* index case in the United States was an elderly patient with leukemia on Orcas Island, Washington, who presented with a pulmonary *C. gattii* nodule in January 2006 (Upton et al., 2007). Since then, the outbreak has expanded into the United States from Canada, and we summarize here the evidence and key findings.

From 2006 to 2010, approximately 70 cases in patients have been reported, and the most complete records are attributable to the efforts of the CDC *Cryptococcus* working group. The cases in humans and animals are shown in Figure A10-1, and the icons represent cases for which we have isolates in the laboratory that have been molecularly analyzed. Two types of isolates are circulating on Vancouver Island: the VGIIa/major genotype, which causes approximately 95 percent of the infections, and the VGIIb/minor genotype, which represents the remaining 5 percent of isolates (Fraser et al., 2005). Both of these genotypes have been found in the environment on Vancouver Island and cause infection in both patients and animals, and both have now emerged within the United States. Of particular interest is a completely novel genotype that has emerged in Oregon: VGIIc, or the novel genotype (Byrnes et al., 2009, 2010). VGIIc has not yet been found in Washington state, Vancouver Island, or anywhere else in the world; thus, it is a completely new emergence from 2005 to 2010 in Oregon.

We can identify genotypes by applying MLST of genetic barcodes throughout the genome. In this technique, coding and non-coding regions of genes are PCR amplified, sequenced, and assigned a unique allele number. The alleles are then color coded and organized in a tabular format in which each line represents a different isolate associated with the outbreak or a global isolate in the strain collection. This allows one to appreciate that there has been what appears to be a large clonal expansion of the VGIIa/major genotype in the region that dominates

³⁶ See contributed manuscript by Bartlett in Appendix A (pages 101–116).

³⁷ See contributed manuscript by Harris in Appendix A (pages 207–225).

the outbreak on Vancouver Island and its expansion into Puget Sound and beyond in Washington state. There are fewer isolates of the VGIIb/minor genotype, but these have been found on Vancouver Island, in Oregon, and most recently in Washington state. The VGIIc/novel genotype has thus far been found only in Oregon and has several, unique alleles not found to date in any other isolates examined from global sources. The CDC has identified one isolate from a patient in Idaho that appears to be closely related to the VGIIc/novel genotype, but it may be the result of travel exposure (DeBess et al., 2010).

Where did these VGII outbreak genotypes originate? The VGIIa/major genotype is indistinguishable across 30 MLST loci and several variable number of tandem repeat loci from the NIH444 strain, which was isolated in the early 1970s from a sputum sample from a patient in Seattle (Fraser et al., 2005). This isolate is the type strain for *C. gattii* and is molecularly indistinguishable from the VGIIa/major outbreak strain at all loci examined thus far. Therefore, the major outbreak strain appears to have been in this geographic region for at least 40 years and possibly even longer. The VGIIb/minor genotype isolates are indistinguishable from isolates from a fully recombining sexual population in Australia (Campbell et al., 2005a,b; Fraser et al., 2005). The VGIIc/novel genotype has thus far only been identified in isolates collected in Oregon, and we have not observed it in a large collection of more than 200 *C. gattii* isolates collected globally. It appears as if the VGIIc/novel genotype either emerged locally in Oregon or, alternatively, it may be present in an undersampled environmental niche that remains to be discovered. Further evidence that this new genotype in Oregon is novel involves haplotype network mapping. In this approach, the MLST alleles are compared and we apply a computer algorithm to predict the ancestral allele. The alleles are then organized into a diagram rooted with the ancestral allele, and alleles derived from it by mutations and genetic drift are indicated with lines and circles. In this analysis alleles that are arisen from the ancestral allele long ago lie closest to the ancestral allele, whereas alleles that arose recently lie distal. The MLST alleles that are private to the VGIIc genotype are distal in these haplotype networks, suggesting that they arose recently. The alleles that the VGIIc alleles are derived from come from isolates that originated from either Australia or South America (Byrnes et al., 2010). This analysis then suggests that those alleles might represent possible sites of origin of at least some of the genetic material in the Oregon VGIIc/novel outbreak isolate.

We have conducted mammalian virulence studies in a mouse inhalation model, both at the Wadsworth Center in Albany with our collaborators Ping Ren, Sudha Chaturvedi, and Vishnu Chaturvedi and also at Duke University (Byrnes et al., 2010; Fraser et al., 2005). The VGIIa/major genotype and the VGIIc/novel genotype isolates are both highly virulent in the mouse model used, whereas the minor VGIIb genotype is considerably attenuated by direct comparison (Byrnes et al., 2010). Isolates collected globally that share many but not all markers with the VGIIa/major genotype are considerably less virulent than VGIIa (Byrnes

et al., 2010). This includes, for example, isolates from both California and South America. Of note, the NIH444 type strain (a VGIIa/major genotype isolate) is also somewhat less virulent than contemporary outbreak VGIIa/major genotype strains, which may reflect either attenuation as a result of long-term storage and passage; variation in virulence even among the VGIIa/major isolates; or unknown genetic differences between NIH444 and the VGIIa/major outbreak genotypes in regions of the genome not yet analyzed. Therefore, it appears as if virulence has increased with the emergence of the VGIIa/major outbreak genotype and the VGIIc/novel genotype from their original source strains.

Several investigators have been addressing why outbreak isolates are more virulent than other genotypes. There have been two prescient studies. Jim Krostad and colleagues at the University of British Columbia in Vancouver have shown that there is less protective inflammation in the lungs of mice infected with the outbreak isolates (Cheng et al., 2009). This may then lead to increased virulence if the host fails to mount a sufficient, protective immune response. In addition, the work from Robin May's lab at the University of Birmingham has described an increased intracellular proliferation rate of the VGIIa and VGIIc outbreak isolates in macrophages and linked mitochondrial function to the robustness of their interaction with host immune cells (Byrnes et al., 2010; Ma et al., 2009). Given that *Cryptococcus* is a pathogen able to grow either outside or inside of host immune cells, an enhanced proliferation rate in the context of the macrophage intracellular milieu may lead to enhanced virulence. These studies are excellent starting points to use to begin to dissect the virulence mechanisms at the interface with host immune cells in the lung.

To summarize, this outbreak was originally restricted geographically to Vancouver Island and then spread to the mainland of British Columbia. Starting in 2006 (and maybe earlier in one case in Oregon in 2005), it emerged within the United States. Thus, there clearly appears to have been a geographic expansion from Vancouver Island across Puget Sound to reach Washington, mostly involving the VGIIa/major genotype. However, there is more diversity in strains from Oregon, involving both the VGIIa/major and VGIIb/minor genotypes observed on Vancouver Island, but also including the novel VGIIc genotype that has not been identified on Vancouver Island. In essence, this looks like an outbreak within an outbreak, which may be of independent origins. It is as though two pebbles have been dropped into a pond at different times, one earlier than the other, and they have generated concentric waves that are now expanding outward and intersecting. There is considerable concern that this outbreak will continue to expand geographically given that there are cases and isolates now from Idaho and California (Personal communication, Shawn Lockhart, CDC Mycotic Diseases Branch, May 2011). There is also a well-documented risk to travelers to the endemic region, who then return to locations around the world with an infection of the VGIIa/major isolate and present with a very unusual fungal infection that is not commonly described in those regions and that might confuse clinicians

(Chambers et al., 2008; Georgi et al., 2009; Hagen et al., 2010; Lindberg et al., 2007).

The Role of Sexual Reproduction in Pathogen Emergence and Outbreaks

The second charge of this article was to consider the role of sexual reproduction in the emergence of pathogens and how it might contribute to adapting to a novel niche. We might start with just a very general question of, why sex? For those investigators who focus on bacteria or other prokaryotes, their mechanisms for genetic transfer typically involve horizontal gene transfer. But for the eukaryotic pathogens, including fungi, parasites, and the oomycete plant pathogens such as *Phytophthora infestans* (the cause of potato blight), their genetic exchange mechanisms involve sexual reproduction. The general theme that emerges for all of these groups of eukaryotic pathogens is that sexual reproduction plays a critical role in their diversity and, in many cases, also in their infection cycles (Heitman, 2006, 2010).

What benefits are conferred by sexual reproduction? This process enables the exchange of genetic information and generates diversity, and it can also purge the genome of deleterious mutations. It also allows these organisms to stay one step ahead of their transposable elements, which might otherwise accumulate to litter the genome.

We know a great deal about the sexual reproduction of *Cryptococcus* because of the pioneering work of June Kwon-Chung at the National Institutes of Health some 30 years ago (Kwon-Chung, 1975, 1976a,b). There are two mating types, called **a** and α (Hull and Heitman, 2002; McClelland et al., 2004). A panoply of signals regulates the interactions of cells of opposite mating type, allowing fusion and completion of their sexual cycle, thereby generating infectious spores. We know from both historic and recent experimental studies that these spores are indeed infectious propagules (Botts and Hull, 2010; Botts et al., 2009; Giles et al., 2009; Sukroongreung et al., 1998; Velagapudi et al., 2009; Zimmer et al., 1984).

Two of the many signals that regulate the *C. gattii* sexual cycle are interactions with plants and extreme desiccation (Xue et al., 2007). As noted by Karen Bartlett, there is more *Cryptococcus* found in the air in Vancouver Island in July when it is very hot and dry (Bartlett, 2010). Indeed, these are also the ideal conditions for stimulating the sexual cycle, at least under laboratory conditions. Thus, *Cryptococcus* may mate more in the hot and dry conditions of July than in other times of the year.

Sex in *Cryptococcus* produces spores, which are infectious. There is also a link of the mating type to virulence that has focused interest on its sexual cycle (Kwon-Chung et al., 1992; Nielsen et al., 2003, 2005a,b; Okagaki et al., 2010). One of the most curious features of this species is that most of the *Cryptococcus* population is just one mating type (α), even though the sexual cycle that was originally defined in the laboratory requires both opposite mating types (Hull

and Heitman, 2002). This fact raised a central conundrum for the entire field of cryptococcal pathogenesis: If there is a well-maintained **a**- α opposite-sex sexual cycle, but the **a** mating type is extremely rare, if not completely absent in many populations, how then can a sexual cycle be an important step of the infectious cycle for this fungus? It might be that it is not important, such that these unisexual populations were just clonally reproducing mitotically as yeasts. However, we discovered that *C. neoformans* can undergo an unusual sexual cycle involving only one of the two mating types (Lin et al., 2005).

The opposite-sex cycle that we have known about for 30 years is depicted in the lower panel of Figure A10-3. Then in 2005, an alternative sexual cycle was reported called unisexual reproduction or same-sex mating, and this is depicted in the upper panel of Figure A10-3 (Lin et al., 2005; Wang and Lin, 2011). This alternative sexual cycle only involves α cells. They can fuse with another α cell from the population, or they can, in extreme cases, fuse with themselves, undergo meiosis, and produce spores.

You might wonder, what would be the point of undergoing sexual reproduction with yourself? There is no genetic diversity to admix in this circumstance. We always think about sexual reproduction as involving two parents with very

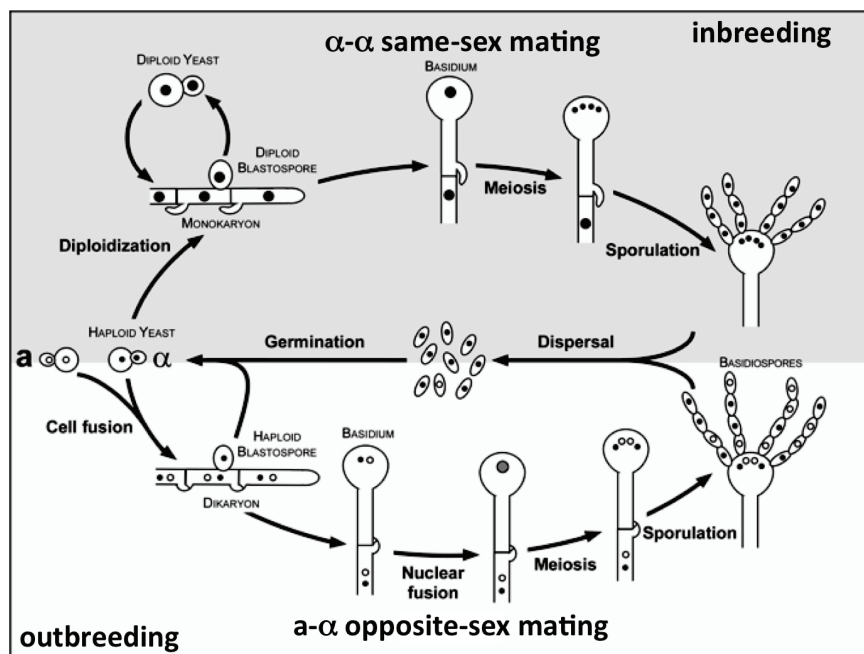


FIGURE A10-3 *Cryptococcus neoformans* can reproduce unisexually and bisexually.

different genetic compositions. It turns out that mating with yourself can also lead to the generation of genetic diversity. Sex itself can serve as something of a mutagen to generate genotypic and phenotypic diversity. This strategy turns completely on its head what we traditionally think of as the primary role of sexual reproduction. An analogy might be the appearance of mismatch repair mutator mutations in bacterial pathogens, which arise to result in the generation of genome-wide mutations and are then swept from the population as a consequence of their concomitant deleterious effects.

As an example, in preliminary studies we isolated 100 progeny produced by this same-sex mating cycle and looked for phenotypic diversity. We have found within this set of just 100 isolates clear examples of azole resistance, temperature-sensitive growth, hyperfilamentous growth, and increased melanin production. By comparison, analysis of 100 progeny produced by mitotic clonal growth yielded no such examples. Many of the phenotypically distinct isolates turn out to be aneuploid in one way or another. This is based on comparative genome hybridization analysis showing that one of these morphologically distinct isolates now has an extra copy of chromosome 10. The presence of an extra copy of a chromosome is termed aneuploidy. We often associate aneuploidy with very deleterious consequences. We do not have to go further than Down syndrome as an example to think about what might be bad about aneuploidy. On the other hand, in the microbial kingdom, aneuploidy can be a rich source of phenotypic diversity. There are well-documented studies in *Candida albicans* that an a special type of chromosome formed by duplicated arms of a chromosome (termed an isochromosome) can form and drive fluconazole resistance (Selmecki et al., 2006, 2008, 2009). Similarly, we also know that azole heteroresistance in *Cryptococcus* is driven by aneuploidy for chromosome 1 (Hu et al., 2008; Sionov et al., 2009, 2010). A variety of other experimental evolution studies have brought to the forefront the idea that aneuploidy might be a driving force for genotypic and phenotypic plasticity (Pavelka et al., 2010; Rancati et al., 2008; Torres et al., 2007, 2010). So this may be one mechanism by which a same-sex mating cycle could engender phenotypic and genotypic diversity in a population without needing a partner that is genetically divergent.

Another way to view same-sex mating is that it is a mechanism for selfing. Selfing/inbreeding is common in fungi and the paradigm is mating type switching in *S. cerevisiae*, which allows mother–daughter cell mating. Same-sex mating is another route to self, but not via mating type switching. It is a question at some level of outcrossing vs. inbreeding. Same-sex mating superimposes much more limited genetic diversity on a well-adapted fungal genome that has run the gauntlet of natural selection. As such, unisexual reproduction may confer one of the benefits of sex (generation of diversity), but at the same time avoid one of its costs (breaking apart well-adapted genomic/genetic configurations). For organisms that are well adapted to a niche, this more limited genetic diversity may better answer subtle changes in selection pressures.

We have alluded to the fact that there are restricted geographic areas where fungal sex is extant. Why is that? Why would you have these little areas and pockets in Africa and that is where they have sex, and in the rest of the world they don't have sex except for the unusual unisexual cycle? At least for *Cryptococcus*, what it looks like is, where they have opposite-sex mating seems to be where there are restricted populations where both mating types still exist. We know the most about this from *C. neoformans*, where there is an extant population that is on a very specific indigenous tree in South Africa, in Botswana (Litvintseva et al., 2011). But everywhere else in the world, you find just the alpha mating type (Hull and Heitman, 2002). So there is a niche in nature where opposite-sex mating is occurring. Until 5 years ago everyone presumed everywhere else it was asexual and mitotic. But what we are coming to appreciate is that there is just a different version of the sexual cycle occurring in these other populations where there is just one mating type.

This is interesting as another classic example is *Phytophthora infestans*. In Mexico and in Peru and other areas of South America, that is where the sexual cycle occurs, new versions arise, and then they are often exported on potato crops. That was the source of the Irish potato famine. But why there is a sexual cycle in such a restricted place is not really clear. *Toxoplasma gondii* might be another interesting pathogen to consider. It only has its sexual cycle in cats and other felids, not in other animals of any sort (Heitman, 2006). So what is it about the gastrointestinal tract of a cat that is so different from a mouse or a human that allows this parasite to undergo its sexual cycle there? The parasite sexual cycles are even more bizarre than some of the fungal ones, and restricted to extreme niches (Heitman, 2006, 2010).

What about the specific example of *C. gattii* sexual reproduction? June Kwon-Chung elucidated the *C. gattii* sexual cycle more than 30 years ago (Kwon-Chung, 1976a,b). Spores were produced in her classic studies via an α - α opposite-sex mating cycle that she defined. We were approached by several of the Australian groups working on the VGI genotypes in the late 1990s, including Wieland Meyer and Dee Carter, and we spent more than 5 years recapitulating this sexual cycle under laboratory conditions. This required a great deal of heroic effort on the part of several individuals in various laboratories, in large part because fecundity can be reduced with long-term passage and storage and because the VGI molecular type that is predominant in Australia is more recalcitrant in mating assays compared to the VGII and VGIII *C. gattii* molecular types. We were finally able to recapitulate a sexual cycle for *C. gattii* involving the formation of dikaryotic hyphae with special cells linking the hyphal cells (fused clamp cells) culminating in the production of basidia and basidiospores, all of the morphological features of the sexual cycle, including meiotic recombination (Campbell et al., 2005a,b; Fraser et al., 2003). Based on this advance, we were able to show that the vast majority of the outbreak isolates from Vancouver Island are fertile in laboratory crosses (Byrnes et al., 2010; Campbell et al., 2005a;

Fraser et al., 2003). These are **a**- α matings that were conducted, but we stress the point that every single isolate that is associated with the outbreak is of the α mating type. No one has found any **a** isolates occurring anywhere on Vancouver Island or in Washington or Oregon.

How might mating occur on Vancouver Island and in the Pacific Northwest in this unisexual population? In previous studies we observed that an association of *Cryptococcus* with plants can stimulate the fungal sexual cycle (Xue et al., 2007). Part of the idea in testing for this phenomenon in the first place was that plants, and more specifically trees, are the environmental niche for *C. gattii* (Heitman et al., 2011). For the plant pathogenic fungus *Ustilago maydis*, which infects corn, the infectious form is the filamentous dikaryotic hyphae produced by the sexual cycle, which is stimulated by interaction with the plant. In fact, *U. maydis* has to be in its sexual form to infect the plant host. We found something very similar with *Cryptococcus*. We were unable to infect a plant efficiently with the haploid yeast, but the filamentous dikaryotic state will infect plants and stimulate a plant defense response (Xue et al., 2007). In turn, if we put seedlings distant from a fungal mating mixture on a plate, the plants secrete small molecules, such as inositol, that stimulate completion of the sexual cycle and production of spores (Xue et al., 2007, 2010). We observed this with seedlings of *Arabidopsis* or Eucalyptus and are now exploring this with Douglas fir because of the link to this indigenous tree species in the Pacific Northwest outbreak. This line of investigation suggests that this may be one niche in nature where the sexual cycle is occurring to produce spores as small airborne infectious propagules.

Another critical line of investigation involves population genetic studies conducted by Dee Carter from the University of Sydney, Australia. She has focused on the sexual cycle that may be occurring in Eucalyptus tree hollows. She discovered that in populations with both mating types, they can engage in opposite-sex mating, but in other tree hollows where only α isolates are found, they are also sexually recombining (Saul et al., 2008). So it seems as if the organism has two extant sexual cycles, one opposite sex and one same sex, and whom you mate with depends on who your neighbors are in the population. All of her studies have focused on the VGI cryptic species, which has very limited genetic diversity and an extremely high level of apparent inbreeding (Campbell and Carter, 2006; Campbell et al., 2005a,b; Carter et al., 2007, 2011). All of the isolates on Vancouver Island associated with the clonal outbreak are of a different cryptic species, VGII. However, based on Dee Carter's studies of VGI sexual reproduction, the presumption is that there may be both forms of sexual reproduction occurring also for the VGII isolates in nature, associated with the environmental niche in trees or plants for the VGII lineage and involving both opposite and same-sex mating depending on where **a** isolates are found in nature.

We have advanced the population genetic analysis in two ways with our global isolates to look for measures of sexual reproduction and recombination occurring in the population. One of these is a simple test called an allele com-

patibility test. The basic premise is that if you cross two strains that differ at two markers, you obtain four types of progeny produced by sexual processes by reassorting two unlinked loci (i.e., AB by ab yields AB, ab, Ab, and aB) (Carter et al., 2011), which we all know from studying Punnett squares. So the multilocus sequence loci can be analyzed as two unlinked alleles in the population. If you find all four possible combinations, this is indicative of sexual reproduction and recombination occurring in the population. By looking at the multilocus sequence markers, we find that these measures of recombination are rampant throughout the VGII global population (Byrnes et al., 2010). In addition, if we just examine the sequences of the multilocus sequence alleles themselves, which represent approximately 1 kilobase of sequence each, we can find examples of hybrid recombinant alleles that implicate isolates as potential parents or potential offspring (Byrnes et al., 2010). A lot of mitotic recombination must be occurring to see evidence of recombination within just a random 1-kilobase sequence in a 20-megabase genome. The potential parents that are identified by this type of analysis originate from South America, Africa, and Australia (Byrnes et al., 2010). These findings suggest that sexual reproduction is occurring globally in multiple environments and locations.

We went on to examine these global isolates for fertility in the lab and documented, by scanning with electron microscopy, the production of meiotic spores, which are the products of sex that may be infectious propagules found in the air on Vancouver Island. The majority of the α strains we analyzed are fertile. We have found a very limited number of **a** strains in the global population. For example, we found 6 out of 200 (~3 percent) are this minor mating type. Five out of six of these **a** strains are fertile in the laboratory, and thus they may be undergoing opposite-sex mating in nature. Because five of these six **a** isolates are from South America, it may be a site in which opposite-sex mating is still extant in the population (Escandon et al., 2007; Fraser et al., 2005; Ngamskulrungroj et al., 2008). We know that *C. neoformans* has also retained extant opposite-sex mating, but that it is geographically restricted to Botswana and South Africa and occurring in the VNB *C. neoformans* lineage (Litvintseva et al., 2003, 2007).

When we return to consider the VGIIb minor genotype and its relationship to global isolates, this provides critical insight. For instance, we looked at 30 MLST alleles, the VGIIb outbreak isolates are completely indistinguishable from isolates from a fertile, unisexual, sexually recombining population in Australia that was identified by Dee Carter and colleagues (Campbell et al., 2005a,b). It suggests that this population may be the ancestral source for the VGIIb/minor outbreak genotype isolates now circulating in the Pacific Northwest. While one might posit just the opposite (transfer from Vancouver Island to Australia), the diversity of the population in Australia supports this as the ancestral rather than the derived population. Thus, the most parsimonious model is that the isolates from the outbreak originated from Australia (Figure A10-4).

Based on a broad comparison of the isolates on Vancouver Island, the VGIIa/

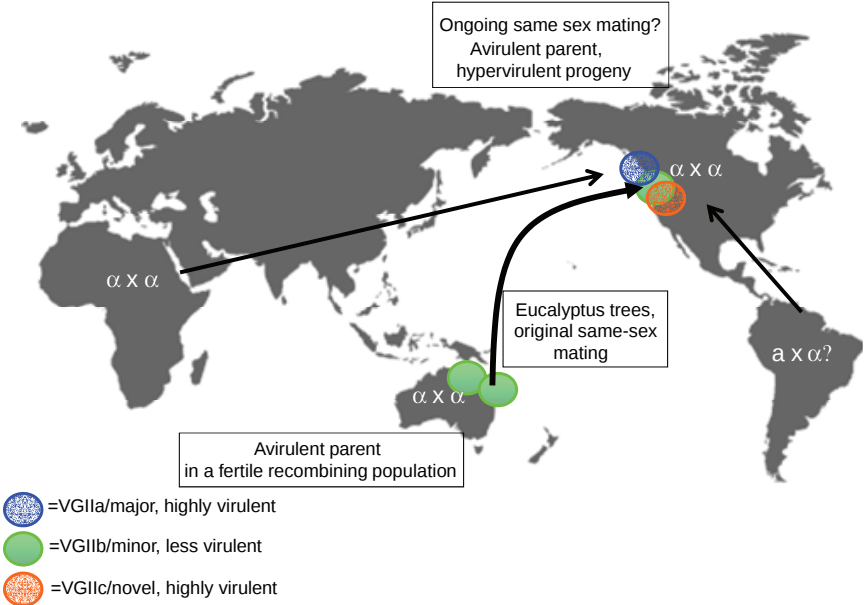


FIGURE A10-4 Sexual reproduction and the origin of an outbreak.

major and the VGIIb/minor genotypes share half of their genetic markers and differ at the other half. So in a very simple model, they might be progeny from a genetic cross (i.e., siblings), or one might be a parent and the other an offspring. In this simple conceptual framework, one could imagine an isolate undergoing mating with an unknown isolate in nature to produce the outbreak isolates. However, the VGIIa/major and the VGIIb/minor genotypes on Vancouver Island are all of the same mating type, α . When we look in detail at the mating type locus, we can see that they are molecularly distinct from each other (Fraser et al., 2005) and are not identical by descent. So, in essence, they have two different ancestries for this region of their genome that dictates their mating type. A more parsimonious model then is that genetic crosses that led to the production of these isolates would have involved two parents with different α mating alleles in a same-sex mating cycle. It is also possible that both types of crosses have occurred (opposite-sex and same-sex mating) and that there has been more than one cross involved here in the genesis of these isolates that are responsible for the outbreak and also for the global isolates to which they are related.

Conclusions and Perspective

To summarize, three molecular genotypes are found circulating in the Pacific Northwest. The most prominent is the VGIIa/major genotype (Figure A10-1 in yellow), and we can date its origin to at least the early 1970s to an isolate from a patient in Seattle. The VGIIb/minor genotype is shown in red and it is molecularly indistinguishable from isolates from a fertile recombining population in Australia. In green is a completely novel VGIIc genotype that has emerged in Oregon. Two of these genotypes (VGIIa, VGIIc) are highly virulent in both macrophage and mouse models (Byrnes et al., 2010).

From where do these VGII isolates originate? We find VGII isolates globally: in Africa, Australia, and South America. But based on available evidence to date, the most parsimonious model is that at least one of these genotypes (VGIIb/minor) came from a population in Australia, given that isolates from the two are indistinguishable (Figure A10-4). In terms of the crosses hypothesized to have been involved in the origin of the outbreak, all of the populations that have been identified are all α . There are no **a** mating types of this lineage that have been found on Vancouver Island, in the Pacific Northwest, or in Australia. The only places where VGII mating type **a** isolates have been found so far are South America and Greece. So it may be that in Australia and the Pacific Northwest that same-sex mating drives diversity in the population, but opposite-sex mating is a possibility in South America. In the context of the outbreak, we also envision that same-sex mating is contributing to the production of infectious spores, which are the aerosolized small particles detected with Anderson air samplers. It is entirely possible that those propagules are desiccated yeast cells, spores, or a mixture of both. Experiments to test if these are actually spores in nature are being planned, and if spores are present in the air on Vancouver Island, it seems likely that they are being produced by same-sex mating occurring in the environment, given that the population is α unisexual.

Disease caused by *C. gattii* vs. *C. neoformans* has been the subject of several reviews, and despite the impression that *C. gattii* frequently causes infection in apparently immunocompetent hosts and commonly presents with focal cryptococcomas in the lung or brain (Chen et al., 2000; Mitchell et al., 1995; Speed and Dunt, 1995), it has been difficult to clinically distinguish between *C. neoformans* and *C. gattii* disease. In fact, the presentation of *C. gattii* infections in Vancouver and the Pacific Northwest outbreak appear similar to *C. neoformans* infections, and disease is not limited to the immunocompetent host. *C. gattii* infections such as those caused by the VGIII molecular type/cryptic species can be even found in HIV-infected individuals in endemic areas (Byrnes et al., 2011; Chaturvedi et al., 2005). Another aspect that has been reported to distinguish these two species is their response to therapy. It has been suggested that *C. gattii* infections require longer therapy (West et al., 2008). However, in vitro susceptibility testing has produced inconsistent results; some investigators have found similar minimum inhibitory concentrations (MICs) whereas others have found higher MICs to azoles for

C. gattii compared to *C. neoformans* (Thompson et al., 2009; Torres-Rodriguez et al., 2008). It is always difficult to be precise in determining the response to therapy when in a normal host; a component of immune reconstitution inflammatory syndrome may influence clinical judgment of treatment failure. A recent study primarily in HIV-infected patients demonstrated that there was no difference in the outcome of *C. gattii* vs. *C. neoformans* infections in a single medical center (Steele et al., 2010). This is interesting and may be due to small numbers, but early results comparing *C. gattii* infections in the Vancouver Island vs. the Pacific Northwest outbreaks suggested a higher mortality in the United States (Kluger et al., 2006). After review of present data on outcomes in the literature, in vitro drug susceptibility studies, and clinical experience, the 2010 Infectious Diseases Society of America Cryptococcal Guidelines support the treatment of *C. gattii* infections in a manner similar to those of *C. neoformans* (Perfect et al., 2010). However, *C. gattii* infections may produce more cryptococcomas in the brain or lung, and chronic conditions such as hydrocephalus and a slow response to antifungal therapy may be prominent features of disease with this species.

Despite the lack of precise differences in *C. gattii* vs. *C. neoformans* disease clinically, which would require immediate identification of the species, there are differences in epidemiology regarding the range of infections. It is also suggested on a pathobiological basis that *C. gattii* may present more with acute disease rather than reactivation disease. This hypothesis is based on (1) antibody studies in children where *C. neoformans*, but not *C. gattii*, antibodies are frequently detected, and (2) the higher percentage of disease that is observed in immunocompetent patients with *C. gattii* (Goldman et al., 2001). It has also been shown that *C. gattii* may induce a different host immune response compared to *C. neoformans* (Cheng et al., 2009). Furthermore, the Vancouver Island outbreak represents an ideal arena to check for genetic susceptibility to *C. gattii* infection because a vast number of individuals were exposed to high numbers of infectious propagules, but only a minority of those exposed was identified with clinical disease. However, it is clear through both animal studies and transplant cases that *C. gattii* infections can reactivate (Dromer et al., 1992; Kluger et al., 2006). Finally, although major virulence factors (melanin, capsule, high-temperature growth) are similar, some of the networks such as the trehalose pathway connecting these virulence factors are different in these two species (Ngamskulrungraj et al., 2009b; Petzold et al., 2006). The possibility that differences in both host and microbe genetics may influence the course and management of infections will drive studies on this unique example of a fungal outbreak in humans as we continue its analysis.

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A11

YEAST INFECTIONS—HUMAN GENETICS ON THE RISE³⁸

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We lead inextricably mycotic lives: yeasts leaven our bread, ferment our wine and beer, and inhabit our skins, mouths, and gastrointestinal tracts; however, not all is harmony. Hippocrates reported aphthous ulcers consistent with thrush in patients with severe debilitation, but it was not until the 1840s that in the newly emerging field of clinical experimental medicine that thrush—as well as other mycotic conditions, including ringworm—was recognized as being caused by transmissible fungi. In 1923 Christine Marie Berkhout named what we now call *Candida albicans*, for the white robe, *toga candida*, worn by Roman senators and senatorial candidates (Emmons et al., 1977). Fungal infections in general, and candida infections in particular, are important markers of innate or acquired immune dysfunction. However, despite the estimated 1.5 million species of fungi in the world, precious few cause human disease, and those that do (in the

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absence of iatrogenic factors) usually cause inapparent or mucocutaneous infection. Therefore, genetic factors in both the pathogen and the host must be key to an understanding of who gets disease and why.

Despite the ubiquity and importance of fungi, little is known about specific human genetic predispositions to them. There are three distinct categories of fungi: filamentous molds (e.g., *aspergillus*, *mucor*, and *trichophyton*), dimorphic fungi (e.g., the endemic mycoses, *histoplasma*, *blastomyces*, *coccidioides*, and *sporothrix*, a non-endemic pathogen), and yeasts (e.g., *candida*, *cryptococcus*, and *trichosporon*). In the absence of iatrogenic factors, visceral infections of filamentous mold occur almost exclusively in patients with chronic granulomatous disease, whose capacity to generate phagocyte superoxide is defective. Severe invasive aspergillosis develops in these patients (who have normal lungs), as do, on occasion, infections with invasive *candida*; interestingly, mucocutaneous candidiasis is not encountered in chronic granulomatous disease. In contrast, development of most other cases of pulmonary aspergillosis that are unrelated to immunosuppression requires previous airway damage, such as bronchiectasis or bullous disease. Dimorphic fungi cause inapparent or relatively mild disease in the vast number of people who are otherwise normal. Severe pneumonia and disseminated disease occur infrequently, as in cases of advanced human immunodeficiency virus (HIV) and in patients with defects of the interferon- γ -interleukin-12 axis, indicating that intracellular killing plays a crucial role in the body's defense against these infections. Mucocutaneous candidiasis, vaginal candidiasis, thrush, and onychomycosis occur in a variety of disparate conditions, ranging from diabetes mellitus to the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome, to HIV, to Job's (hyper-IgE) syndrome. Our recent identification of mutations in signal transducer and activator of the transcription 3 gene (*STAT3*) as the cause of Job's syndrome unexpectedly provides a link to the important articles by Ferwerda et al. (2009) and Glocker et al. (2009) in this issue of the *Journal*.

Whereas mucocutaneous candidiasis has long been recognized as a consequence of profound lymphocyte dysfunction, or lymphopenia, it occurs in Job's syndrome in patients with a relatively normal number of functioning lymphocytes. Mutations in the gene encoding transcription factor *STAT3* impair numerous pathways, including the generation of interleukin-17-committed T lymphocytes (Th17 cells). Th17 cells fill a gap in previous knowledge about lymphocyte-mediated killing: If Th1 (interferon- γ -producing) lymphocytes are controlling intracellular infection and Th2 (interleukin-4-producing) lymphocytes are directing antibody-mediated protection against extracellular infection, which cells protect the exposed mucosa and epithelium? Among the products of Th17 cells is interleukin-22, which synergizes with interleukin-17 in the epithelial synthesis of cationic antimicrobial peptides, such as defensins. *STAT3* is also central to the induction and signaling of itself, interleukin-17, and interleukin-22 (Fig. A11-1) (Minegishi et al., 2009).

We encounter most microbes first at epithelial surfaces through cell-surface

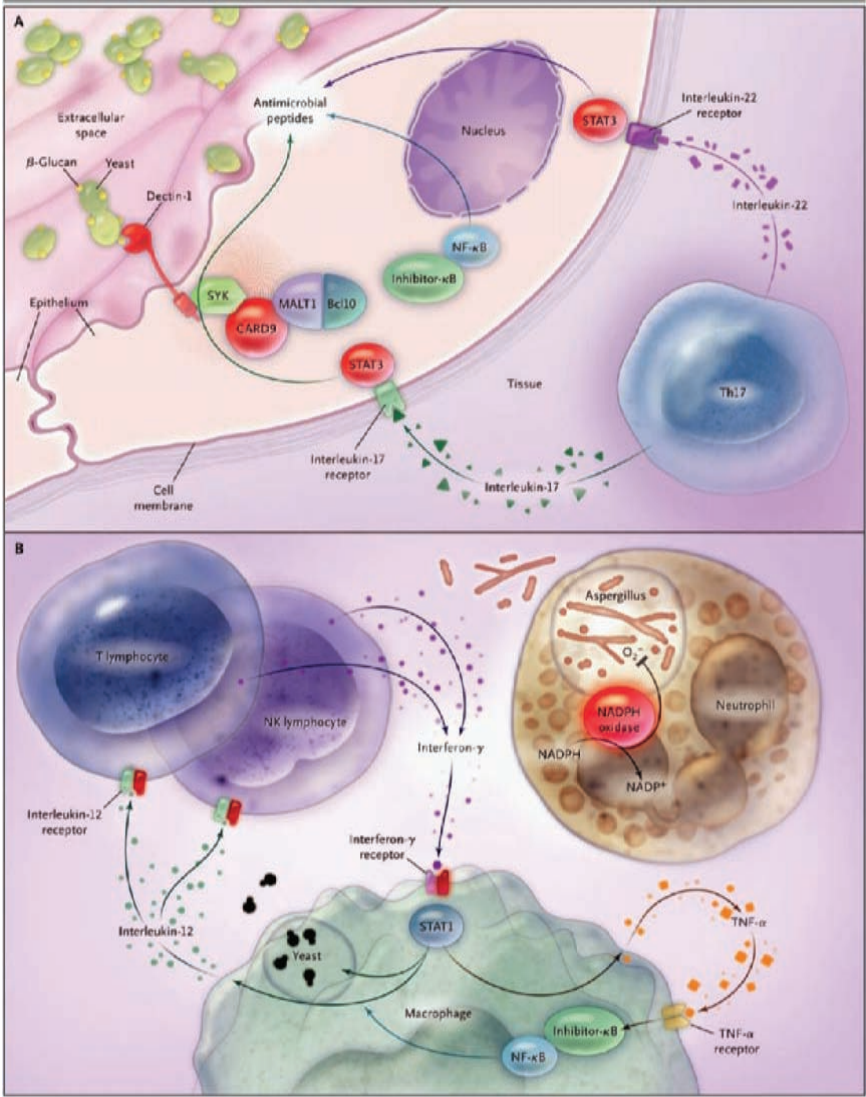


FIGURE A11-1 (facing page). Mechanisms of Fungal Sensing and Control. The β -glucan on the budding yeast forms of candida bind to dectin-1 on epithelial cells and phagocytes, leading to activation of the CARD9 signaling complex (along with Bcl-10-MALT1) (Panel A). This in turn produces cytokines that help drive CD4+ T lymphocytes toward the Th17 phenotype, a STAT3-dependent process. Th17 lymphocytes elaborate interleukin-17 (augmenting neutrophil production and recruitment) and interleukin-22, which are synergic in the STAT3-dependent production of antimicrobial peptides by epithelial cells. The generation of superoxide by the phagocytic NADPH oxidase system is crucial for the killing of filamentous molds such as aspergillus (Panel B). Interactions between macrophages and lymphocytes are required for the control of intracellular infections such as the dimorphic fungi. In response to fungal infection, macrophages release interleukin-12, which acts on its cognate receptor on T lymphocytes and natural killer (NK) lymphocytes. Interleukin-12-stimulated lymphocytes release interferon- γ , which acts on macrophages through STAT1 to kill intracellular fungi. Interferon- γ also augments tumor necrosis factor α (TNF- α), which acts in part through the nuclear factor κ B (NF- κ B) to kill fungi and drive inflammatory responses. Molecules with identified mutations relevant to fungal susceptibility are shown in red.

receptors. These receptors couple the binding of specific microbial components to signal transduction pathways, which results in local and systemic immune responses, including activation of the central activator of inflammation, nuclear factor κ B. Specific immunodeficiencies involving receptor molecules and their signaling have been characterized for herpes simplex virus encephalitis, *Neisseria meningitidis*, and *Streptococcus pneumoniae* (Beutler, 2009). As expected, the closer the defect is to the initial contact with the pathogen, the more likely it is that the susceptibility will be microbiologically narrow. In contrast, defects that occur at the convergence of several pathways tend to be broader and more severe. Dectin-1 is the cell-surface receptor for β -glucan, a major component of the budding yeast-cell wall, and its signaling travels through a series of molecules, including caspase-recruitment-domain (CARD) protein 9, leading to activation of nuclear factor κ B.

Ferwerda et al. identified a Dutch family with impaired in vitro responses to β -glucan. The spectrum of disease was limited to nails and mucosa. Interestingly, the ages at clinical disease presentation were 10 to 12 years for the homozygous daughters but 40 and 55 years for the heterozygous mother and father, respectively, suggesting both hormonal and gene-dose effects. Generation of interleukin-6 and interleukin-17 was impaired only in response to the dectin-1 ligand, β -glucan. The specific stop codon the authors identified in dectin-1 is remarkably common in some parts of Africa and Europe (allele frequency, 3 to 7%), suggesting that unrecognized forces maintain it in populations. Glocker et al. identified an extended Iranian family with predominantly mucocutaneous but also fatal candidiasis of the central nervous system caused by mutations in the critical dectin-1 signal transduction molecule, CARD9, impairing both dectin-1 signaling and Th17 production. CARD9 also funnels a variety of other cell-

surface and intra-cellular signals, including the p38 mitogen-activated protein kinase (MAPK) and Jun N-terminal kinase (JNK) pathways, possibly accounting for its greater clinical severity as compared with isolated dectin-1 deficiency. The CARD9 mutation appears to be rare, and its rarity is commensurate with its severity.

Important caveats concerning both reports are that the key manifestations are mucocutaneous, whereas the functional studies were performed on leukocytes. It remains to be proven whether the key mechanisms in these cases of severe candidiasis consist of impaired dectin-1 signaling at the epithelial level or impaired leukocyte activation of epithelium, mediated through cytokines such as interleukin-17.

The long and largely happy coexistence of humans and fungi has necessitated the existence of ways to detect and control fungi, keeping them in their place. Apparently, these pathways are different for candida, aspergillus, and mucor. Although we do not yet understand the entire conversation between leukocytes and epithelium, with these two reports we have now overheard some of the key words that will enable us to listen more thoughtfully.

No potential conflict of interest relevant to this article was reported.

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A12

THE INCREASED RISK OF GLOBAL WHEAT RUST PANDEMICS: PUTTING YELLOW RUST INTO PERSPECTIVE

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Infectious diseases of humans are receiving great attention due to their direct and immediate impact on human mortality (King et al., 2006; WHO, 2008),

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whereas the diseases of crop plants are subject to much less publicity although they are threatening crop productivity, food security, and thereby the livelihood of billions of people around the world. Currently, wheat rust fungi are among the top 10 constraints for food production in many parts of the world, such as sub-Saharan Africa, East Asia, and South Asia (FAO, 2010; Waddington et al., 2010), mainly due to their epidemic potential and transboundary spread by wind (Brown and Hovmøller, 2002).

According to U.S. Department of Agriculture statistics, global wheat production was generally lower in 2010 compared to the previous year (USDA, 2011). The reduced productivity was caused by several factors, such as unfavorable weather conditions, resulting in large-scale flooding, droughts, and bushfires in different parts of the world, as well as severe yellow rust epidemics (USDA, 2010).

Rust diseases have been recognized as being harmful to wheat since ancient Greece. Theophrastus of Eressus (371–286 BC), the founder of botany and a pupil of Aristoteles, noted in his book *ΠΕΡΙ ΦΥΤΩΝ ΑΙΤΙΩΝ* (*The Causes of Plants*) that some plants of barley were more susceptible to the rusts than others, the most susceptible denoted “Achilles Barley,” and he recognized the importance of sowing in due time to reduce rust diseases on different crop plants such as wheat, barley, peas, and beans (Theophrastus, 1990). Wheat rust may be caused by three plant pathogenic fungi, *Puccinia graminis* (Leonard and Szabo, 2005), *Puccinia triticina* (Bolton et al., 2008), and *Puccinia striiformis* (Hovmøller et al., 2011). The corresponding diseases are termed black (stem) rust, brown (leaf) rust, and yellow (stripe) rust, respectively (synonymous terms in brackets) (Figure A12-1).

The three wheat rust fungi are biotrophic and heteroecious, meaning they generally require a primary wheat host for asexual reproduction and an alternate host to complete sexual reproduction. *Berberis* spp. have been known to serve as an alternate host for stem rust for more than two centuries. For instance, Schøler (1818), who was a school teacher in Denmark, demonstrated a link between stem rust on barberry and some cereals via repeated infection experiments. His results started a so-called “barberry quarrel,” where the usefulness of barberry eradication programs was discussed. Later, de Bary (1865, 1866) demonstrated the complete life cycle of the stem rust fungus. However, the problems related to barberry adjacent to cereal fields were observed by farmers in Europe much earlier, with the first local laws against barberry being implemented in France in the 17th century and in the Americas in the 18th century (Roelfs, 1982). Surprisingly, the discovery of barberry as an alternate host for yellow rust infecting wheat and *Poa* grass, respectively, was not made until very recently (Jin et al., 2010). The alternate host of *P. triticina* depend on the primary host: Isolates from cultivated wheat and wild emmer have *Thalictrum speciosissimum* (in the *Ranunculaceae*) as alternate host, whereas several species in the *Boraginaceae*, such as *Anchusa aggregata*, *Anchusa italica*, *Echium glomeratum*, and *Lycopsis arvensis*, may



FIGURE A12-1 Typical macroscopic symptoms of rust infections on adult wheat plants. *Puccinia striiformis* Westend (yellow/stripe rust) [left photo], *Puccinia triticina* (brown/leaf rust) [center photo], and *Puccinia graminis tritici* (black/stem rust) [right photo].

serve as alternate host for *P. triticina* from wild wheat and rye (Anikster et al., 1997). Although this paper deals with all three wheat rusts, the primary focus is yellow rust, which has spread at unprecedented scales in recent years and caused severe epidemics even in areas where the disease was previously non-significant or absent (Hovmøller et al., 2010).

Yellow Rust Epidemiology

Wheat rusts are highly epidemic on susceptible cultivars in a rust-favorable environment. In the past, yellow rust was considered most harmful in cool and wet climates, whereas leaf rust and stem rust epidemics were favored by warmer temperatures. They are all characterized by passive spreading of airborne urediniospores carried by the wind, potentially across hundreds of kilometers (Hovmøller et al., 2002; Kolmer, 2005; Leonard and Szabo, 2005; Zadoks, 1961). Since 2000, epidemics of yellow rust in particular have accelerated in many areas (Hovmøller et al., 2010). In the United States, annual losses due to wheat yellow

rust exceeded 1 million metric tons over several years, and in each of the years 2003 and 2010 they mounted to 2.4 million tons (Long, 2000–2010), despite increased and widespread use of agrochemicals already in 2003 (Chen, 2005). In China, yellow rust is considered the most damaging disease on wheat, which is grown on more than 20 million hectares (Wan et al., 2004). In three yellow rust epidemic years, annual losses varied between 1.8 and 6 million tons; in 2002, losses of 1.3 million tons were recorded and 1.9 million tons were saved by treating at least 6 million hectares with fungicides (Wan et al., 2004). Yellow rust epidemics reached record levels in northern Africa in 2009 (Ezzahiri et al., 2009) and also in central and western Asia (Mboup et al., 2009), where more than 90 percent of important wheat varieties were susceptible to the disease (Sharma et al., 2009). Areas particularly affected by yellow rust epidemics in 2009 and 2010 are illustrated in Figure A12-2.

New genetic variability of the pathogen, resulting from natural population dynamic forces such as mutation, recombination, and migration, may be followed by host-induced selection, which is a major driver of changes in gene and genotypic frequencies of biotrophic plant pathogens (Hovmøller et al., 1997).

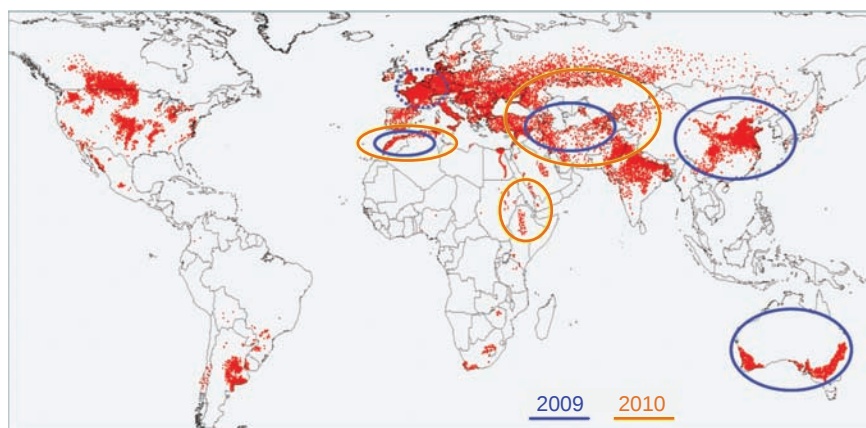


FIGURE A12-2 Map indicating the distribution of global wheat production and regions of recent yellow rust epidemics. Each red dot is equivalent to 20,000 tons of wheat grain production. The blue and orange circles indicate some of the areas in which severe yellow rust epidemics on wheat were reported in 2009 and 2010. Yellow rust severity varied greatly from field to field within the highlighted areas, depending on susceptibility of actual host cultivar, local environmental conditions, pathogen race distribution, and disease management practices. The epidemics in Northwest Europe in 2009 (dotted circle) were restricted to relatively few cultivars of wheat and triticale.

SOURCE: Adapted from Trethowan et al. (2002). Dr. Dave Hodson, Food and Agriculture Organization of the United Nations, Rome, is acknowledged for kind permission to reprint the map.

In agricultural systems, where crop cultivars with similar or identical resistance specificities are cultivated across large areas, the role of selection is greatly enhanced (Bayles et al., 2000; Hovmøller et al., 1997; McDonald and Linde, 2002). Therefore, crop varieties that are resistant when first deployed in agriculture may become susceptible after some years in cultivation. This phenomenon, which is often referred to as the boom-and-bust cycle, has been reported in numerous cases for the wheat rusts (e.g., Bayles et al., 2000; Chen, 2005; Enjalbert et al., 2005; Hovmøller, 2001; Wellings, 2007). The boom-and-bust cycle is closely related to resistance genes in the host and two categories of pathogen traits, virulence and aggressiveness.

Virulence and Aggressiveness

The yellow rust fungus is specialized on the cereal host at both species and cultivar levels (Hovmøller et al., 2011). The former refers to variation in the infection and reproduction capacity of the pathogen on different host genera (e.g., wheat, barley, rye, triticale), whereas the second level of specialization occurs within the host genus, that is, at the cultivar level. The latter is typically based on the interaction between host resistance gene products and pathogen avirulence gene products in a gene-for-gene relationship (Flor, 1956). Mutation in the avirulence gene may result in virulence, which can be defined as the qualitative ability of the pathogen to cause disease by compromising a matching resistance gene in the host. In case the host cultivar possesses more than a single resistance gene, the virulence phenotype, representing a specific combination of different virulences, becomes critical in determining the fate of host susceptibility (e.g., Hovmøller and Henriksen, 2008; Johnson, 1992; Line and Qayoum, 1992). The virulence phenotype can be resolved from the outcome of a race analysis, which is carried out by inoculating a rust isolate onto a set of host differential lines carrying known resistance genes, for details (see, e.g., Hovmøller and Justesen, 2007a).

The study of race dynamics in *P. striiformis* populations has often been an important part of early warning and yellow rust strategies in Europe (Hovmøller and Henriksen, 2008; Johnson, 1992), North America (Chen and Penman, 2005; Line and Qayoum, 1992), China (Wan et al., 2007), and Australia (Wellings, 2007). Since 1967, where a national race survey was established in the United Kingdom (Johnson, 1992), similar programs were established in Germany (Flath and Bartels, 2002), France (De Vallavieille-Pope et al., 1990), and Denmark (Hovmøller, 2001). In recent years, European data on *P. striiformis* virulence dynamics have been made publicly available via the Eurowheat database at www.eurowheat.org, which also describes options for disease control, such as cultural practices and fungicide efficacies (Jørgensen et al., 2010). Systematic race surveys were initiated in the United States in the late 1960s after a series of severe yellow rust epidemics on wheat (Line and Qayoum, 1992). Systematic race surveys have also been implemented in China (Chen et al., 2009; Wan et al., 2004),

India (Prashar et al., 2007), Australia (Wellings, 2007; Wellings and McIntosh, 1990), and South Africa (Boshoff et al., 2002).

In addition to the virulence phenotype, pathogen aggressiveness, which is a quantitative measure of the ability of a virulent isolate to cause disease on a susceptible host plant, is a key determinant for the rate of pathogen spread and evolution. Some of the recent epidemics since 2000 have been ascribed to the emergence of yellow rust strains with increased aggressiveness and tolerance to warm temperatures (Hovmøller et al., 2008). In this context, strain was defined as a group of *P. striiformis* isolates sharing both molecular marker phenotype and virulence phenotype. For instance, Milus et al. (2009) demonstrated a significantly increased aggressiveness at both high and low temperatures, compared with isolates of pre-2000 races from North America and Europe (Milus et al., 2009). At the low-temperature regime, gradually changing from 10°C (night) to 18°C (day), the aggressive strains produced more than 70 percent more spores per infected leaf area compared to reference isolates from before 2000. At the high-temperature regime (12°C at night to 28°C during the day), isolates of the aggressive strains produced approximately 150 percent more spores per infected leaf area. Milus and colleagues (2009) concluded that the aggressive strain had most likely enhanced the yellow rust epidemics in North America and contributed to the spread of yellow rust into areas that were previously considered too warm for yellow rust epidemics. The aggressive strain detected in North America was indistinguishable from a strain detected as exotic incursion in Australia in 2002 (Hovmøller et al., 2008; Wellings et al., 2003). In Europe, a nearly identical strain was first detected in 2000, whereas first appearance of this strain in the Red Sea area and in western and central Asia is unknown (Hovmøller et al., 2008). According to time and area, these observations may be comparable to the famous panglobal spread of the Irish potato famine fungus *Phytophthora infestans* (Goodwin et al., 1994).

The distinction between virulence and aggressiveness may be subject to controversy because virulence toward a resistance gene with minor effects may be manifest by increased disease progress and spore production. These are the same variables used to measure aggressiveness. However, in case of virulence, there should be evidence for a significant host–pathogen interaction, whereas in the case of aggressiveness, the host–pathogen interaction is ideally non-significant. This implies that for a range of host cultivars with varying levels of rust susceptibility, they should rank the same for aggressive and non-aggressive isolates.

The Need for Coordinated Global Action

The number of foreign incursions of wheat rust races has increased significantly in recent years. Park et al. (2010) reported nine foreign incursions of wheat stem rust and wheat leaf rust into Australia between 1925 and 2005. Of these seven had occurred since 1969. Hovmøller et al. (2011) documented at least six

exotic incursions of yellow rust at continental scales since 1979, often resulting in the spread of yellow rust epidemics to new regions or continents. Scherm and Coakley (2003) noted that the rate of exotic pathogen invasion in the United States had increased from about five instances per decade from 1940 to 1970 to more than three times that number during the 1990s. This increasing rate of incursions of the yellow rust fungus may partly be ascribed to the emergence of new, highly aggressive rust strains combined with increasing human travel and commerce. However, regardless of the reasons for the increasing spread, the situation escalates, emphasizing the relevance of pathogen surveys covering larger areas and a need for global coordination. The use of crop cultivars with similar or identical resistance specificities across large wheat-growing areas supports this conclusion.

The accelerating wheat yellow rust epidemics requires coordinated multinational action to complement the ongoing initiatives of the Borlaug Global Rust Initiative (www.globalrust.org) to fight the multivirulent strain of wheat stem rust, known as Ug99 (Northoff, 2007, 2008). The speed by which threatening new strains can be diagnosed and reported widely is essential, and the precision by which traits such as virulence and aggressiveness are diagnosed is another bottleneck (Hovmøller et al., 2011). Precise diagnosis of both traits require pure and correct identification of the seed stocks of standard differential wheat varieties used for the race assays, genetically pure pathogen isolates, and well-defined experimental conditions for temperature, humidity, light, and plant nutrition, as well as trained staff who can assess and interpret the virulence phenotype and aggressiveness. The acknowledgment of these challenges led to the establishment of a Global Rust Reference Center (GRRC) in 2008 targeting yellow rust, supported by the International Center for Agricultural Research in the Dry Areas (ICARDA), the International Maize and Wheat Improvement Center (CIMMYT), and Aarhus University (Denmark) (Hovmøller et al., 2010). GRRC is accessible for receiving yellow rust samples on a year-round basis, which is a major advance complementing the capacities of existing regional and national rust diagnostic laboratories. At the Borlaug Global Rust Initiative (BGRI) Coordination Meeting in Syria in September 2009 (Dold, 2009), a proposal was made to extend the activities of GRRC to all wheat rust fungi, that is, yellow rust, brown rust, and stem rust.

The long-term aims of a Global Rust Reference Center would be the tracking of new wheat rust incursions and assessment of ongoing pathogen evolution at global scales, supplying data to online early-warning systems for farmers, breeders, and policy makers, like the current “Rust Spore” monitoring system run by the Food and Agriculture Organization of the United Nations (FAO, 2010). Another main activity would be to assist in training of students and junior scientists in rust pathology, and maintenance and extension of a unique world wheat rust collection to facilitate resistance breeding efforts. The activities should complement ongoing regional and national survey activities and research efforts

by BGRI partners, national counterparts, and advanced research institutions, resulting in stronger global efforts to mitigate the consequences of the inherent pathogen dynamics. At present, the activities at GRRC are limited to race analysis of relatively few pathogen samples per year in addition to training activities. To become sustainable in the long term, GRRC must contain a considerable portfolio of activities to reduce vulnerability due to potential changes in staff, management, and political awareness.

The identification of sources of resistance from cultivated and wild crop relatives to wheat is probably the most urgent task to reduce the threats posed by the wheat rusts, including yellow rust. Plants have an immunity system with several layers. Race-specific resistance provides protection only during one or few steps of the infection process and is therefore considered vulnerable to pathogen evolution whereas partial resistance, based on several or many sources of resistance, is considered more durable. Nevertheless, breeding for increased partial and non-host resistance protecting wheat against a broad spectrum of pathogen species and races can be done by large-scale field screening of thousands of breeding lines by applying targeted pathogen species and strains.

Prospects for New Research

The serious impacts and characteristics of wheat yellow rust epidemics should remind society about the vulnerability of global food production and the need to understand the interactions between agricultural crops, their pathogens, and the environment. Until recently, investigations of the basic biological and molecular mechanisms of yellow rust evolution have been hampered by the biotrophic lifestyle of yellow rust, precluding the use of molecular tools used for fungi that can be cultured on artificial media, and by the lack of an experimental system for genetic studies. These limitations may soon be overcome by the significant advances of molecular technologies, new insights into pathogen biology, and the cloning of host resistance genes, which were achieved in recent years. Molecular markers are probably the best established tools for resolving recent as well as past dispersal and evolutionary events in *P. striiformis* (Hovmøller and Justesen, 2007b; Hovmøller et al., 2008; Mboup et al., 2009). Next generation sequencing is currently the most promising tool for the identification of genetic changes related to virulence in fungal pathogens (Stergiopoulos and de Wit, 2009). A Danish–British genome sequencing initiative was started in 2009 (Walter et al., 2009), followed by a more comprehensive U.S.-led initiative (Broad Institute, 2009) aiming to resolve the whole genome sequence of *P. striiformis*. Such knowledge will most certainly speed up the discovery of genes involved in pathogenicity. The recent discovery of barberry as sexual host for *P. striiformis* (Jin et al., 2010), which has solved a century-old mystery about the yellow rust lifecycle, represents another major breakthrough that can serve as the basis for the development of an experimental system allowing classical genetic studies.

Functional characterization of isolated rust genes is still a great challenge because of the biotrophic lifestyle, which is hampering genetic transformation of *P. striiformis*. The identification of sources of resistance in wheat, especially for resource-poor areas in Africa and Asia, is probably the most urgent task to reduce the threats posed by wheat rust pathogens. However, this will require a long-term commitment and combined efforts of breeders, pathologists, and biologists to keep pace with the wheat rusts, which can evolve rapidly to compromise genetic resistance of the wheat host (Hovmøller and Justesen, 2007b; Wellings and McIntosh, 1990).

Concluding Remarks

The fight against infectious crop diseases has become a collective responsibility and requires a collective investment with a global, long-term political and scientific commitment. Such a global network will need to establish the physical and human resources to support the progress of wheat rust management technologies, that is, pathogen monitoring and resistance breeding, training programs for farmers and pathologists, and scientific progress. Hence a major priority of a global network should be to link researchers and plant breeders (1) with each other and with appropriate partners in resource-poor countries that are affected most by sudden wheat rust epidemics, and (2) with political authorities to allow for rapid actions. The establishment of the BGRI in 2006, inspired by Dr. Norman Borlaug, the pioneer of the Green Revolution and the front leader of cereal rust resistance breeding, represents a major milestone in this respect. The real challenge is to ensure sustained activities beyond ongoing, short-term projects. In the long term, surveillance and prevention measures of wheat rust epidemics will only be successful if performed on a coordinated, global scale. Borlaug used to say: “Rust never sleeps”—and events of recent years have shown how right he was.

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A13

**FUNGAL PATHOGENESIS IN PLANTS AND ANIMALS:
SIMILARITIES AND DIFFERENCES***Barbara Howlett*⁴¹**Introduction**

Although the vast majority of fungal species do not cause disease, ones that affect plants are responsible for significant economic losses (Skamnioti and Gurr, 2009). In the past couple of decades, fungal diseases of humans have become an increasing threat, especially for people who are immunologically compromised (Sexton and Howlett, 2006). Consequently there has been a rapid explosion in knowledge about fungal pathogenesis of animals and this has been taken up by people studying plant pathogenic fungi. Pathogenesis involves the interaction of two partners with input from the environment, a concept described as the “disease triangle” in plant pathology. The “damage-response” concept developed for animal pathogens emphasizes that the outcome of an interaction is determined by the amount of damage incurred on the host (Casadevall and Pirofski, 2003). These concepts are useful reminders of the complexity of the interaction and the interdependence of host and pathogen.

Tools to Study Fungal Pathogenesis

The large number of fungi with sequenced genomes and recent advances in genetic manipulation of fungi are leading to an improved understanding of mechanisms associated with disease. Comparative genomics is being used to identify candidate genes involved in disease. Amplification of particular gene families within a genome is consistent with lifestyles: For instance, many plant pathogens have large gene families that encode enzymes to degrade the cuticle and cell wall, which coats plant cells, while animal pathogens (e.g., *Coccidioides immitis*, the cause of valley fever) have gene families encoding enzymes that degrade proteins of the skin, which is usually the initial barrier to invasion (Sharpton et al., 2009). Transcriptomics can show which genes are turned on; microarrays have been used extensively and many datasets are publicly available (Cairns et al., 2010). Proteomic analysis of culture filtrates provides information about secreted proteins (secretome) that are accessible to the host and thus may play a role in the interaction; this approach has been applied to identify such proteins of *Leptosphaeria maculans*, the blackleg pathogen of canola (Vincent et al., 2009).

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The generation of fungal mutants via tagged random insertional mutagenesis allows identification of novel genes involved in disease, while targeted gene knockout or gene silencing allows functional analysis of candidate pathogenicity genes. Analyses of human pathogenic fungi generally rely on cell lines and animal models with different immunosuppression regimes. By contrast, plant pathogens can be studied directly on their hosts. Many more fungal species infect plants compared to animals and thus more plant fungal systems than animal fungal systems are studied, but with a shallower focus. Plants obviously can be manipulated with fewer ethical issues than those associated with animal experimentation. Findings from model systems can often be applied to other host–pathogen interactions. The best studied plant fungal system for ascomycetes is rice and *Magnaporthe oryzae* (cause of rice blast) (Ebbole, 2007) and for basidiomycetes is maize and *Ustilago maydis* (cause of corn smut) (Brefort et al., 2009). Model animal fungal systems include immune-suppressed mice and *Aspergillus fumigatus*, *Candida albicans*, or *Cryptococcus spp*; recently amoeboid and non-vertebrate animal model systems, particularly the insect *Galleria mellonella*, have been used (Mylonakis et al., 2007). General similarities and differences between fungal pathogens of plants and animals are described in Table A13-1 and are discussed in more detail later in this chapter.

The Invasion and Disease Process

The disease process can be considered as a series of consecutive steps beginning with recognition between host and fungus, penetration of the host, colonization and avoidance of host defenses, development of disease symptoms, and finally fungal reproduction and dispersal. These steps are developmentally regulated, and fungal mutants can be generated that are arrested at particular stages (for review see Sexton and Howlett, 2006). The mutated genes are often referred to as pathogenicity genes and are broadly categorized as to where they appear to act in the disease pathway. This is an arbitrary classification for convenience. Clearly there are pathogenicity genes with pleiotropic effects at many steps in the pathway, as some processes (e.g., recognition, pH regulation, oxidative burst, signaling) occur several times. Pathogenicity genes of fungi that attack plants have been reviewed recently (Van de Wouw and Howlett, 2011). Interestingly the steps related to symbiotic relationships between plant and fungi are similar to those involved in pathogenesis; thus the classification of plant–fungus interactions as pathogenic or symbiotic is indistinct.

Fungal inoculum of plants is usually spores (sexual or asexual) that then germinate on the surface of the plant. Animal pathogenic fungi can exist in forms such as yeasts, conidia, or hyphal fragments. Some such fungi undergo dimorphic switching. In some cases, the yeast form is more pathogenic than the hyphal phase, while in others the converse is true. A key class of hydrophobic proteins, hydrophobins coat the fungal cell wall, which is composed generally of

TABLE A13-1 General Similarities and Differences Between Fungal Pathogens of Plants and Animals

Feature	Plants	Animals
Number of fungal pathogens	Many	Few
Importance	Cause 25–30% crop losses	Usually affect immunocompromised animals
Lifestyle and environmental niche	Often biotroph; necrotroph; also saprophytes	Often soil saprophytes; not obligates
Experimental systems	Many; often shallow focus	Few; different host cell and immunosuppression regimes of non-humans
Host specificity	Often host species or cultivar specificity	Sometimes species specific, not genotype specific
Mode of entry into hosts	Via stomata, or breaching surface barriers via enzymes or pressure created by infection structures	Via inhalation, ingestion, or wounds in skin
Dispersal	Between hosts	Not usually between hosts
Reproduction	Sexual and asexual	Usually asexual (ascomycetes)
Overall pathogen requirements	Often complex set of physical and molecular barriers to overcome	Inocula small enough to enter host, survive at 37°C and avoid immune responses

chitin, a polymer of β 1,4 N-acetyl glucosamine, β 1,3 glucans, as well as other polysaccharides and proteins.

Plants have more complex physical barriers to invasion than animals do. Epidermal cells on the plant surface have a cuticle comprising epoxy fatty acids, and a cell wall that is composed of a matrix of proteins and interacting carbohydrates, including β 1,3 and β 1,4 glucans such as cellulose (Carpita and Gibeau, 1993). By contrast the animal surface, the stratum corneum, is much thinner and the cells are highly keratinized (Proksch et al., 2008). Fungi enter animals by inhalation, ingestion, or wounds. Basically, for a fungus to be an animal pathogen, its major features include being small enough to enter tissue, able to survive at 37°C, and able to evade host immune responses (Sexton and Howlett, 2006). Indeed, the temperature optimum for many fungi is significantly lower than 37°C, which has been proposed as an explanation for why relatively few fungi are important pathogens of mammals (Robert and Casadevall, 2009). The mammalian characteristics of maintenance and close regulation of body temperature are proposed to have a selective advantage in conferring resistance to many fungal pathogens (Bergman and Casadevall, 2010).

Fungi enter plants via the stomatal apertures, where air exchange occurs; by digesting the cuticle and cell wall with hydrolytic enzymes; or by developing infection structures, appressoria, which accumulate high concentrations of glycerol and puncture the surface due to high turgor pressure (Van de Wouw and Howlett, 2011). As mentioned above, fungi enter animals through the skin or via inhalation or wounds; an exception is *Histoplasma capsulatus*, which enters the host via receptor-mediated endocytosis (Woods, 2003).

The first basal or innate layer of defense is very similar in plants and animals. It involves binding of pathogen associated molecular patterns (PAMPs) to pattern recognition receptors on the host membrane surface. This triggers signaling pathways that induce a range of defense responses, including production of reactive oxygen species and sometimes programmed cell death. This response is termed “pathogen triggered immunity.” PAMPs common to plant and animal pathogenic fungi include wall carbohydrate fragments, such as chitin oligosaccharides or β 1,3 glucans. As well as conservation among PAMPs, there is also conservation in structural domains of Pattern Recognition Receptors (for review see Zipfel, 2009).

In plants often the responses triggered by innate basal immunity are not strong enough to stop pathogen invasion. Consequently a second round of recognition occurs involving effector-triggered immunity (Jones and Dangl, 2006). Often there is a “gene for gene” interaction between avirulence (effector) genes in the pathogen and resistance genes in the plant, such that the pathogen is unable to attack host genotypes with the corresponding resistance genes. Thus there is usually a high degree of host specificity with plant diseases, with only particular varieties (genotypes) of a single species being susceptible. In contrast to plant diseases, most animal diseases do not display host genotype specificity, although some fungi only cause disease in certain animal species. Apart from innate basal immunity, the immune system of mammals is very different from that of plants. The responses that come into play if the pathogen is not stopped by the innate basal immunity response include the innate complement system, circulating cells such as phagocytes that can internalize and destroy pathogen cells, and adaptive antibody-mediated defenses (Speth et al., 2004).

Fungal plant pathogens have a range of lifestyles and nutritional requirements. Many have a saprophytic phase, and some are obligate, surviving only on their hosts. Some (biotrophs) require hosts to be living, while others (necrotrophs) kill plant tissues. Some fungi are both biotrophs and necrotrophs at different stages during growth *in planta*. Some fungi such as mycorrhizae and endophytes colonize plants in a symbiotic relationship deriving carbon from photosynthesis by the plant and often conferring drought tolerance on the plant. Fungi that infect animals often have a saprophytic lifestage in the soil, and few if any are obligate.

After invasion, fungi then need to colonize the host, derive nutrition, and avoid or subvert host defense responses. In many plant–pathogen interactions, defense responses include the hypersensitive response, whereby an oxidative burst

by the plant generates reactive oxygen species associated with the programmed cell death of host cells. This can arrest pathogen growth, particularly that of biotrophs. Necrotrophic fungi can subvert this defense process to derive nutrition from the dead host tissue. Toxins produced by necrotrophic fungi also kill plant tissue. However, they are generally not often important disease determinants. This is also often the case for secondary metabolite toxins produced by fungi that attack animals. Their role may be to protect fungi against predators such as insects, nematodes, and amoebae during their saprophytic growth phase in the soil (Kempken and Rohlfs, 2010).

To complete its lifecycle, a fungus must reproduce and exit the host to find another host. Many animal pathogenic ascomycetes do not have a sexual phase. However, the sexual cycle of basidiomycetes such as *Cryptococcus* spp. is extremely important in virulence, as discussed by Heitman.⁴² Both animal- and plant-pathogenic fungi reproduce mitotically within the host. Although plant-to-plant transmission of fungal disease is very common, direct transmission of fungal pathogens between mammalian hosts is unusual. In plant pathogens, conidia (vegetative spore) production usually occurs after infection has been established and lesions have developed. Conidia are spread from plant to plant by wind or in water droplets. However, many pathogens of mammals are transmitted by inhalation as hyphae or as arthroconidia, in dust and wind (e.g., *C. immitis*) or soil. Increasingly, biofilms, whereby a community of microorganisms attaches to a solid surface, mediate dispersal of fungi in hospital environments (Ramage et al., 2009).

A Fungal Pathogen That Infects Plants and Animals

Given some of the similarities in strategies that fungi use to cause disease in plants and animals, it is perhaps surprising that few organisms have been reported to infect both plants and animals. A single isolate of *Fusarium oxysporum* f. sp. *lycopersici* can infect both plants and animals. The animal experimental system involves injection of the tail vein of immunodepressed mice and the plant system involves incubation on intact tomato roots (Ortoneda et al., 2004). Mutants in several genes of *F. oxysporum* f. sp. *lycopersici* have been tested for their virulence in the animal and plant models. A mitogen activated protein kinase gene (*fmk1*) and an Rho1 GTPase, which are both involved in signaling, are not required for virulence in mice, but are essential for virulence in tomatoes. In contrast, the transcription factor *PacC*, which mediates the environmental pH signal, and the photosensor WC-1, which interacts with a light-sensitive transcription factor, WC-2, are necessary for full virulence in mice, but are not essential for virulence in tomatoes (Martínez-Rocha et al., 2008; Ortoneda et al., 2004; Ruiz-Roldan et al., 2008). These findings highlight redundancy in some signaling pathways

⁴² See contributed manuscript by Heitman in Appendix A (pages 226–248).

TABLE A13-2 Fungicides Used to Control Plant and Animal Diseases

Fungicide Group	Target	Plants	Animals
Azoles	Ergosterol biosynthesis in fungal membrane	Widely used	Widely used
Echinocandins	β 1,3 glucan synthesis	Not used	New drug
Polyenes: Amphotericin B	Ergosterol biosynthesis	Not used	Yes
Nikkomycin Z	Chitin synthesis CHS I	Not used	Trialed against <i>C. immitis</i>
Strobilurins	Mitochondrial cytochrome bc complex	Used but resistance can develop: G143A mutations in cytochrome bc	Not used; toxic
Bion	Salicylic acid analog: Mimics systemic acquired resistance in plants	Used in horticulture; expensive	Not effective as dependent on plant defense responses

and the complexity of these interactions. It will be interesting to compare global transcriptional analyses of tomato and mouse tissue infected with this fungus to further identify networks and signaling components regulated in response to interaction with two extremely different host types.

Control of Fungal Diseases

Animal diseases are controlled mainly by a well-functioning immune system of a potential host. There are no approved vaccines against fungi, and fungicides often are not efficacious (Ostrosky-Zeichner et al., 2010). Plant diseases are controlled by a combination of approaches. These include management of inocula; for instance, removing infected stubble (crop trash) at the end of the growing season; using fungicides; and introgressing (breeding) resistance genes, often from related species, into varieties. For diseases of both types of hosts, a range of fungicides is employed; choices depend on toxicity and cost/benefit ratio (Table A13-2). Azoles target ergosterol biosynthesis in the fungal membrane and are the most common group of fungicides used for diseases on both types of hosts. Echinocandins, which target synthesis of β 1,3 glucans in the fungal cell wall, are now being used to combat fungal diseases of humans, but would not be effective against fungal plant pathogens given that plant cell walls also have β 1,3 glucans. Nikkomycin Z, which targets chitin synthesis, is being used to control *C. immitis* infections (Ostrosky-Zeichner et al., 2010),⁴³ while strobilurins,

⁴³ See article by Galgiani in Appendix A (pages 196–207).

which target mitochondrial cytochrome bc complex, are deployed against plant diseases, but resistance to them can develop and these molecules have a degree of toxicity toward animals (Bartlett et al., 2002). Bion, which is a salicylic acid analogue, can induce systemic acquired resistance in some plants (Beckers and Conrath, 2007). This molecule is used more in horticulture than broad-acre grain crops due to its cost.

Deploying resistance genes is an important strategy in controlling plant disease. When corresponding avirulence genes are mutated or deleted, the fungus is not recognized by the plant, and infection ensues. Thus resistance is overcome, often resulting in severe yield losses. Some fungi can more readily overcome resistance than others; such fungi are deemed to have “high evolutionary potential” and they usually outcross prolifically, producing large numbers of wind-borne sexual spores as inocula (McDonald and Linde, 2002). These properties enable such fungi to adapt to selection pressures imposed by extensive sowing of crop varieties with resistance conferred by single genes. Thus the frequency of virulent isolates will increase and resistance in the plant can break down. An example of such a breakdown of resistance occurred in 2003 when resistance in canola to blackleg disease caused by *L. maculans* broke down (Sprague et al., 2006). This resulted in up to 90 percent yield losses in regions of Australia, costing farmers up to \$20 million (B. J. Howlett, unpublished). The resistance had been conferred by a canola gene named *Rlm1*. The corresponding avirulence effector gene, *AvrLm1*, has been cloned and is located in a very “plastic” part of the *L. maculans* genome (Gout et al., 2006).

Recently the genome sequence of this fungus has been acquired (Rouxel et al., 2011). The fungus has a unique structure. More than one third comprises repetitive elements composed of degenerated transposons. Gene-rich regions with high GC content alternate with gene-poor regions with high AT content with sharp boundaries between them. Disease-related genes such as effectors are interspersed in the gene-poor regions among repetitive DNA. This organization contributes to the readily changeable nature of the fungal genome and enables effector genes to be readily gained, lost, or mutated (Rouxel et al., 2011; Van de Wouw et al., 2010). Markers are now available for several other avirulence effector genes (Fudal et al., 2007; Parlange et al., 2007), and thus the frequency of virulent isolates within fungal populations in the field that are virulent toward a particular resistance gene can be monitored. This information allows farmers to rotate the disease resistance genes in the canola varieties that they sow from year to year. This strategy, which can be applied to other plant diseases, can help prevent outbreaks and maximizes the duration of effectiveness of resistance genes.

Closing Remarks

The development of fungal diseases of plants and animals has many parallels. A fungus must overcome many hurdles to successfully invade a plant and cause

disease. There is a continuum from disease to damage to symbiosis with plant–fungal interactions. The most important properties of a human pathogen are to be able to survive at 37°C and to avoid the immune responses. The use of fungicides to control disease is made on the basis of specificity and after cost/benefit analyses. Such decision making about a broad acreage crop is very different from that about a human patient. An important strategy to control plant disease is to incorporate resistance genes. However, virulence toward this resistance can be selected for, due to the high evolutionary potential of some fungi. Thus resistance genes have to be managed (rotated) by farmers to maximize longevity of resistance.

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A14

CLIMATE, GLOBALIZATION, AND TRADE: IMPACTS ON DISPERSAL AND INVASION OF FUNGAL PLANT PATHOGENS

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Climate change is likely to become a major issue in plant pathology over the coming years. In this overview, we provide recent evidence for this statement. Moreover, we point out the importance for future plant disease management of climate change interactions with other global change drivers, such as increased long-distance trade. Recent advances in aerobiology, together with new molecular tools used in landscape and geographical genetics, can help in addressing the challenges posed by an increasing number of emerging plant diseases worldwide. There is increasing evidence that climate change will be a key issue in how plant pathogens will affect food security and ecosystem health.

Less knowledge is available on the potential impacts of climate change on biological control of exotic fungal plant pathogens. Network theory is a promising tool to improve biosecurity in the face of the increased volumes of traded plants coupled with climate warming. Although there are now many reviews of the literature on the topic of climate change and plant diseases, there is a need to keep up with the rapid development of the subject.

Introduction

The emergence of fungal plant pathogens can occur as the result of at least three processes: (1) the evolution of new genotypes within a pathogen population that is endemic to a habitat or location (e.g., through genetic recombination); (2) the introduction of an exotic pathogen genotype into a new, receptive habitat or location; and/or (3) the natural selection of new pathogen genotypes from an endemic population as a result of changes in host genotype, host species, or external pressures (e.g., environmental conditions, fungicide applications, changes in plant cultural practices). Emergence is a function of introduction (via dispersal or evolution), establishment (i.e., adaptation to the habitat or location), and spread

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(i.e., dispersal via natural or human-mediated mechanisms). Weather variables, including wind patterns and storms, and the global trade in plants and plant products facilitate the dispersal of plant pathogens and thus provide opportunities for the emergence of fungal plant pathogens. Further, weather is a primary regulator of invasion into new habitats and locations by plant pathogens. Consequently, climate change may provide unprecedented opportunities for the emergence of fungal plant pathogens through the dispersal of fungal pathogens to new locations and/or through habitat modifications for both pathogens and plants.

Climate, globalization, and trade are major drivers of the dispersal and invasion of fungal plant pathogens, affecting agricultural and horticultural crops, plantation and forest trees, and plants in natural/semi-natural environments. Patterns of weather, notably large-scale air movements, have been implicated in long-range dispersal of several economically important pathogens between and within continents. In some cases, continental dispersal of plant pathogens is tracking and catching up with previous movements of crop plants, in turn accompanying human population migrations that occurred over centuries, sometimes millennia. The rate of movement of humans, crop plants, and plant pathogens has intensified markedly in recent decades with the increased globalization of the world economy. Expansion of trade pathways and technological innovations make possible the production, harvesting, storage, marketing, shipping, and air freight of food crops, ornamental plants, and plant products to an extent that previously was not possible (Figure A14-1).

From a broad perspective, climate and global change are drivers of large-scale ecological perturbations that facilitate novel “biomixing” and “ecological fitting” (Agosta et al., 2010). These phenomena can lead to rapid host switching, the emergence of hybrid pathogens, and invasion of new infectious diseases and pests (Brasier, 2008; Palm, 1999). Changes in plant phenology occur in a variety of ways, depending on species and geography. Such changes impact on plant interactions with fungi in general (Gange et al., 2011; Kausarud et al., 2010) and with fungal pathogens in particular (Grulke, 2011; Marçais et al., 2009). The consequence is that multiple species at many sites need to be studied in order to understand and predict regional change and impact (Ibanez et al., 2010). An alternative strategy has been to focus on multiple drivers of global change on a single species (Baeten et al., 2010; Matesanz et al., 2009; Paajanen et al., 2011). Global environmental change arises from CO₂ enrichment, increased nitrogen deposition, climate shifts, biotic interactions, and land use change (Tylianakis et al., 2008). These factors have pervasive effects on antagonistic and mutualistic interactions between plants and fungi and in some cases increase the severity of pathogen infection while weakening mutualisms. Interactions must be expected between tree genetic diversity, variation in phenology, resistance to defoliators and fungal pathogens, increased CO₂, and ozone concentration affecting tree growth and mortality; some of these interactions have been reviewed by Pautasso (2009).

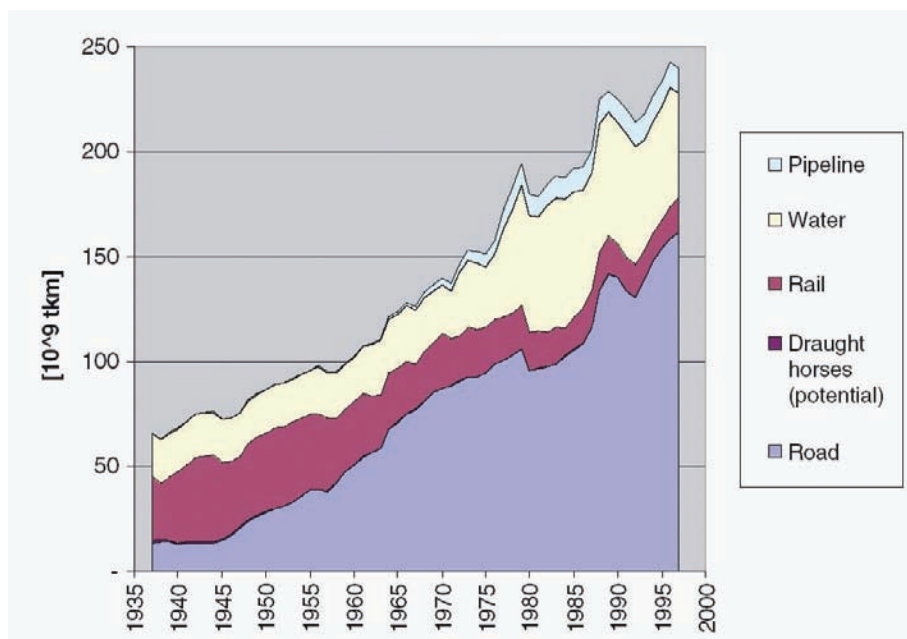


FIGURE A14-1 The increase in goods (10^9 tons $\times$ km) moved in the United Kingdom from the 1930s to the 1990s.

SOURCE: Schulz (2004).

In this overview we consider the impact of climate, globalization, and trade on dispersal and invasion of fungal plant pathogens in the broader ecological context described above. We make a distinction between dispersal that is mediated by natural means, mostly atmospheric, and that which is mediated by human intervention of one form or another. This is partly a matter of convenience: we recognize that at some scales of dispersal both means can be important. With respect to pathogen emergence, dispersal is ineffective without establishment, spread, and persistence—the elements of invasion.

Natural Dispersal of Fungal Plant Pathogens

Fungal spores that escape the boundary layer of crop canopies can be transported over long distances subject to their biophysical characteristics and meteorological conditions. The study of such transport belongs firmly in the domain of aerobiology, a discipline that owes much of its development to the effect of aero-allergens on human health (Dallafor and Sesartic, 2010). An account of an early pioneer of aerobiology, Philip Gregory, and the link with fungi of agricultural and human health concern, can be found in Lacey et al. (1997). Weather has

significant effects on the incidence of aero-allergens, including the abundance and biodiversity of spores of the fungal plant pathogen *Alternaria alternata* (Magyar et al., 2009). Under conditions predicted by climate change, changes in planting practices and modified crop management may be required to keep allergen concentrations under control (Beggs, 2010).

Even if climate change is becoming important in allergen aerobiology (D'Amato and Cecchi, 2008), vegetation normally will be the main source of fungal spores in the atmosphere. Thirty-two genera of fungi arising from vegetation sources were found across both cultivated and urban areas in three regions in Egypt, with a clear association with weather conditions and many implications for the spread of human and plant diseases (Awad, 2005). Fungal concentration in the atmosphere may not be the best indicator of health risk, which may be more associated with the predominant aero-allergen present (Awad, 2005). The effects of meteorological factors on atmospheric dispersal, in biophysical terms relevant for plant pathogens (including viruses and bacteria), was provided in a recent review (Jones and Harrison, 2004).

In some cases, the dispersal of fungal plant pathogens has been modeled explicitly using biophysical principles. A recent example has been the dispersal of *Phakopsora pachyrhizi*, causing soybean rust (Andrade et al., 2009). The American continent was free of *P. pachyrhizi* until 2001. After its introduction in Paraguay, the pathogen rapidly became established throughout Bolivia, Brazil, and Argentina, possibly due to a combination of large-scale cultivation of the plant host, international movement of infected material, and long-distance natural dispersal. Urediniospores of this fungus have been shown to remain viable long enough to be able to travel hundreds of kilometers (Savage et al., 2010). In 2004, Asian soybean rust was reported in the continental United States (Goellner et al., 2010). The fungus now overwinters in warm southern U.S. locations. Spore escape was modeled and combined with a standard large-scale transport model to forecast spore deposition over U.S. soybean production areas. Canopy turbulence and canopy porosity were found to be key determinants of spore escape.

Some effort has been made to test the validity of transport models (Skelsey et al., 2009). Spijkerboer et al. (2002) evaluated the Gaussian plume model (GPM) for predicting and describing spore dispersal over a potato crop. The main purpose was prediction of *Phytophthora infestans*, but for experimental purposes they used a commonly used fern spore. They concluded that the GPM was not applicable in risk assessments, unless combined with site-specific information at the source, such as spore escape in relation to wind speed.

Other more empirical approaches can also be used for specific purposes. High-speed imaging showed that, by synchronizing the ejection of thousands of spores, ascomycete fungi such as the pathogen *Sclerotinia sclerotiorum* form an air flow that carries spores around intervening obstacles to atmospheric currents and new infection sites (Roper et al., 2010). Mundt et al. (2009) used simple empirical relationships based on the inverse power law to describe the spread of

plant diseases such as wheat stripe rust, wheat stem rust, potato late blight, and Southern corn leaf blight. Much of the earlier literature on this approach is cited by the authors. They found that the estimated power law parameter varied little over five orders of magnitude on a distance scale. Evidence was found to support the hypothesis that disease advances through accelerating, rather than constant, waves. Integration of (unmanned) aerial measurements of spore concentration at various distances from the source with simulation of spore flight trajectories is important to develop reliable decision support systems to predict risk of disease spread, as shown for *Phytophthora infestans* in potato fields in Virginia (Aylor et al., 2011; Techy et al., 2010).

A feature of recent work on dispersal has been the integration with population genetics aspects of pathogen diversity (Hovmøller et al., 2008; Montarry et al., 2010). This can operate at the level of host specialization and biotrophy and the ways in which wind dispersal acts as a survival mechanism—what has been termed “Oases in the desert” (Brown et al., 2002). Equally, the strongly stochastic nature of long-distance dispersal can lead to founder effects in the pathogen population (Brown and Hovmøller, 2002). In such cases, pathogen genotypes that successfully establish in new regions and/or on new cultivars may be different from those at the source—the so-called founder effect. Such effects were found in the migration of the fungus causing black Sigatoka of bananas, *Mycosphaerella fijiensis*, from Southeast Asia to Africa and Latin America (Brown and Hovmøller, 2002; Rivas et al., 2004). In both of the new regions founder effects were present in the new invasions. Unresolved for this pathogen is the relative importance of air-dispersed ascospores (probably limited) and the movement of infected plant material (largely unrecorded). The fungus *Corynospora cassiicola* has a wide geographical range in the tropics and subtropics and many plant hosts. Common fungal lineages were widely distributed geographically, indicating long-distance dispersal of clonal lineages, but also previously unrecognized genetic diversity involving some degree of host specialization on some hosts (Dixon et al., 2009). The advent of modern molecular tools in epidemiology provides a step change in both the tracking of dispersal of novel fungal genotypes and in risk assessment of emerging fungal diseases in plants and in animals (Gladieux et al., 2011; Moslonka-Lefebvre et al., 2011).

Climate and Plant Diseases

Weather and climate generally have major impacts on diseases caused by fungal plant pathogens. This topic has had extensive historical coverage that it is not possible to cover in this overview. What is more significant and of immediate concern is how climate change will impact the distribution and severity of diseases caused by known pathogens and the emergence of new invasive pathogens. This topic has also had its fair share of literature reviews: recently, e.g., in the context of structural change in the international horticultural industry (Dehnen-

Schmutz et al., 2010); the evolution of the phytosanitary regulatory framework (MacLeod et al., 2010); forest health and adaptive management (Parks and Bernier, 2010); the impacts on plant health and carbon sequestration in Australia (Singh et al., 2010); cool season grain legume crops and their diseases (Thomas, 2010); urban trees and their pathogens (Tubby and Webber, 2010); diseases in tropical plantation crops (Ghini et al., 2011); the disease triangle and changes in plant phenology (Grulke, 2011); rice diseases and pests (Haq et al., 2011); diseases of food crops (Luck et al., 2011); the geographical distribution of plant pathogens (Shaw and Osborne, 2011); and plant pathogens in Sweden (Roos et al., 2011).

Here, we refer to climate change to include trends in air composition as well as trends in global warming. Air composition in terms of SO₂ concentration has been shown to be associated with the relative prevalence of two important fungal pathogens of wheat (Fitt et al., 2011), a finding made possible by the application of molecular analytical tools to archived plant material from the long-term Broadbalk experiments at Rothamsted Research in the United Kingdom. Elevated atmospheric CO₂ and ozone concentrations decreased the incidence of downy mildew disease in soybean, but increased the severity of disease caused by *Fusarium virguliforme* (Eastburn et al., 2009). Changes in precipitation and temperature resulted in increased disease severity for both diseases, and there were indirect effects due to treatment effects on canopy structure and leaf age. Similar kinds of results were obtained in studies of four pepper diseases (Shin and Yun, 2010). Elevated CO₂ and temperature treatments increased the rate of progress for two bacterial diseases, but not for a stramenopile disease (*Phytophthora capsici*) or a fungal disease (*Colletotrichum acutatum*).

The relationship between climate change, plant diseases, and food security (Chakraborty and Newton, 2011) considers international cooperation and integrated solutions, including disease management issues, to be essential to meet the food demands of the growing world population. Plant breeding for climate-related traits such as drought avoidance or tolerance (Khan et al., 2010) must also take into account disease resistance. The arable sector will be critical in this respect, with mitigation and adaptation strategies with respect to plant disease control likely to become a key area (Fitt et al., 2010). Because of the variation in crop growth and pathogen environmental requirements, geographical divides in crop yield and productivity may become more pronounced (Butterworth et al., 2010). Climate change will also impact on the incidence of mycotoxins in food (Russell et al., 2009), a much-neglected topic in relation to food security and human and animal health.

Forest health is one area where the impact of climate change and biological invasions is providing a clear signal (Kliejunas et al., 2008; Sturrock et al., 2011; Woods et al., 2010). In turn, emerging forest diseases under climate change can lead to a positive feedback by reducing the carbon stocks of affected forests (Peltzer et al., 2010), as seen in British Columbia with the developing *Dothis-*

troma needle blight epidemic following the devastating mountain pine beetle outbreaks. The resilience of northern boreal forests to rapid climate change can be questioned (Chapin et al., 2010), as can forest establishment under conditions of permafrost thaw where fungal pathogens may affect seedling survival (Camill et al., 2010). In forest tree nurseries in Finland, rust, powdery mildew, and other fungal leaf diseases are already causing more problems because of climate warming (Lilja et al., 2010). A more optimistic outlook due to improved adaptive management practices is presented with respect to white pine blister rust (Hunt et al., 2010), where white pines have broad ecological ranges and are less likely to be maladapted thus succumbing to the disease, and hence may be more resilient in the long term. Similar issues will need to be faced with regard to urban trees (Tubby and Webber, 2010) and Mediterranean forests (Attorre et al., 2011; La Porta et al., 2008), where the impact of non-native insect pests and fungal pathogens introduced through trade pathways (see later sections) is already being observed.

The above discussion concerns general issues regarding climate change and plant diseases. There have been many accounts of global/climate change impacts on diseases caused by specific fungal plant pathogens in recent years. A selection of these is cited with summary comments in Table A14-1.

Climate and Beneficial/Biocontrol Fungi

Compared with studies on plant pathogenic fungi, there have been relatively few studies on the effects of climate change on beneficial plant-associated fungi. There has been some consideration of mycorrhizal associations and endophytic fungi, but little on tritrophic mycoparasitic interactions (Singh et al., 2009). In a review of the results of 135 studies, Compant et al. (2010) found that elevated CO₂ had a positive influence on the incidence of arbuscular and ectomycorrhizal fungi, whereas the effects on endophytic fungi were more variable. Effects of temperature were idiosyncratic, with positive, neutral, and negative effects equally common. Plant growth-promoting fungi (as with bacteria) positively affected plants subject to a degree of drought stress. Considerable research has been done with tritrophic interactions among arthropod pests and their natural enemies (Thomson et al., 2009), including fungal entomopathogens; similar research is lacking for biocontrol of plant pathogens (Ghini et al., 2008). The expectation must be that prediction will be difficult because of the indirect and direct effects on biocontrol fungi, unless there is a good understanding of tritrophic interactions (Thomson et al., 2009). Biological control of weeds using fungi is simply a case of a plant pathogenic fungus being used for a beneficial purpose. *Cirsium arvense* is a troublesome weed with world-wide distribution. Climate change will exacerbate invasion and persistence of the weed and make biological control using a variety of pathogenic fungi more difficult (Tiley, 2010).

TABLE A14-1 Selected Papers Illustrating the Effects of Climate and Global Change Factors on Specific Pathogen–Host Systems

Pathogen	Host	Reference	Comments
<i>Valsa melandiscus</i>	Alder	Worrall et al. (2010)	Previous oscillation period in damage of ~21 years likely to dampen with warmer summers and with no period of recovery
<i>Erisiphe necatrix</i>	Grapevine	Pugliese et al. (2010)	Increase in CO ₂ concentration did not affect incidence possibly due to increased photosynthesis with higher CO ₂ and temperature
<i>Phytophthora citricola</i>	Beech	Fleishmann et al. (2010)	Elevated CO ₂ and low N supply enhanced susceptibility, but host compensation followed
<i>Cercospora</i> spp.	Red bud and Sweet bud	McElrone et al. (2010)	Incidence/severity under elevated CO ₂ greater with above average rainfall and temperature (one species), but mitigated by higher photosynthetic efficiency
<i>Leptosphaeria maculans</i> and <i>L. biglobosa</i>	Oilseed rape	Stonard et al. (2010)	Geographic variation in species may be exacerbated by climate change with more damaging species expanding in range
<i>Didymella rabiei</i>	Chickpea	Frenkel et al. (2010)	Isolates infecting crop and wild Cicer show different adaptation to high temperature, with the potential for hybrids infecting both hosts over a broader temperature range
<i>Phakopsora pachyrhizi</i>	Soybean	Del Ponte and Esker (2008)	Integrating epidemiological and meteorological factors suggest no restricted overwintering areas in Brazil
<i>Seiridium cardinale</i>	Mediterranean cypress	Zocca et al. (2008)	Pathogen is associated with insect vectors, which are able to reach the range margin and thus the continuous threat of arrival in the expanding range
<i>Cronartium ribicola</i>	White pine	Frank et al. (2008)	Pathogen arrived in New Mexico in ~1970 with upper flow synoptic classification indicating early June 1969 most favourable
<i>Fusarium</i> spp.	Wheat	Xu et al. (2008)	Environmental conditions differentially affect infection and colonization process and the comparative abundance of six toxigenic species in the head blight disease
<i>Fusarium</i> spp.	Maize	Horvath (2003)	Damage by beetles provides conducive growing conditions for the toxigenic fungi, with feeding behavior changing under drought conditions

Human-Mediated Dispersal of Fungal Plant Pathogens

Thus far we have considered natural dispersal and invasion of fungal plant pathogens, where the main drivers are weather variables, or more generally climate. Although pathogens have always accompanied humans and their crops over the centuries, most notably with the historical migrations of human populations (Guillemaud et al., 2011; Money, 2007), there have been instances where crops moved to new areas have escaped, at least temporarily, the pathogens endemic in the original distributional range of the crops (Mitchell and Power, 2003). Equally, there are cases where crops grown in a new environment have been exposed to novel pathogens. These movements have taken place over centuries with periods of time for adaptation (assisted through plant breeding) or for the pathogen to reencounter the host in a new environment. With the globally connected world that now exists, these time scales are much shorter—decades or, in some cases, much less. The consequence has been the emergence of new fungal plant pathogen species or levels of subspecific variants that were previously unrecorded. For example, since the publication of Erwin and Ribeiro's 1996 Handbook on the genus, 39 new species of *Phytophthora* and two species of hybrids have been formally described (Ersek and Ribeiro, 2010). It is unlikely that this increase is due solely to the advent of modern molecular taxonomic techniques. More likely it is due to the ability of this genus to adapt to new hosts in new environments, through encounters made possible by new pathways.

Less spectacular has been the historical accumulation of non-indigenous forest pests in the United States, where some 450 insect and pathogen species have colonized since European settlement (Aukema et al., 2010). Some 16 pathogenic species have caused substantial damage to trees. This finding is more in keeping with analyses over 10 taxonomic groups of alien species (including fungi), which suggested a historical legacy going back at least a century (Essl et al., 2011). This result would imply that Britain is more at risk for invasion of new exotic species than other European countries (as has happened, for instance, for *Phytophthora ramorum*), given its historical links to its Commonwealth (Figure A14-2). However, even where that is the case, the corollary is that the impact of current global activity will be even more manifest in the decades to come (Crooks, 2005).

Dispersal Through Trade Pathways

Even in a country with an efficient and visible plant quarantine service, such as Australia, there are problems in defining what is present and what is absent (Hyde et al., 2010). These authors make a case for a reinventory of Australia's plant pathogens and consider five fungal groups in which what were thought to be species were in fact species complexes. Without this level of discrimination concerning what is in the country, it will not be possible to operate an effective quarantine and plant protection service. The often tortuous relationship between plant quarantine and trade barriers can be a problematic political issue (MacLeod



FIGURE A14-2 The world in 1897, with British possessions marked in red.
SOURCE: Cambridge University Library available at Wikimedia Commons.

et al., 2010; Perrings et al., 2010). Emerging plant diseases can be seen as negative externalities deriving from the international trade in plants (Lansink, 2011). The removal of trade barriers, however, can be beneficial and in some cases desirable. For example, seed trade legislation was designed primarily to protect trade and return royalties to contemporary plant breeders. Increasingly, the importance of exploiting the genetic diversity present in cereal land races has been recognized, but to exploit their use fully, changes in legislation will be required (Newton et al., 2010). Also the genetic diversity of target plant pathogens should be used in building comprehensive collections to allow efficient, reliable, and specific diagnostic and detection tools in the national and international trade (Barba et al., 2010).

The Sanitary and Phytosanitary Agreement of the World Trade Organisation specifies that countries cannot regulate against unknown pests, yet many invasive alien forest insects and pathogens were new to science when first recorded in a new environment (Britton et al., 2010). To counter this, effective surveillance systems are required to facilitate early detection; these are lacking in many nations. Britton et al. (2010) propose a global network of sentinel plantings based in historical gardens and arboreta to enable early detection and rapid response

to such incursions. To support early detection, diagnostic capability and capacity are needed to provide rapid and accurate identification of emerging or introduced pathogens. Progress has been made in establishing diagnostic capability in rural areas of some developing nations and improving diagnostic capability in some developed nations (Boa, 2007; Miller et al., 2009; Stack, 2010).

Mitigating the nursery stock pathway (for forest trees and ornamentals) for undescribed pathogens will be extremely difficult. In general, analysis has been lacking on how the structure of trade pathways affects the spread of plant pathogens in the nursery trade. Pautasso et al. (2010c) analyzed the hierarchical structure in terms of the proportions of producers, wholesalers, and retailers involved in the trade. Despite the many uncertainties associated with commercially sensitive trade information, it was concluded that disease management options should concentrate on the middle tier of the nursery hierarchy, particularly in the absence of hubs (superconnected nodes). If hubs are present, then control is better targeted at them because (*ceteris paribus*) the higher the correlation between links in and out of nodes, the lower the epidemic threshold (Moslonka-Lefebvre et al., 2009). In addition, the number of outgoing links from the starting node of simulated epidemics in small-size directed networks (a realistic assumption for plant trade among countries and within regions) is strongly correlated with epidemic final size at equilibrium (Pautasso et al., 2010b). These analyses were motivated by the outbreaks of the emerging plant pathogen *Phytophthora ramorum* but have broader generality for systems where susceptibility and infection are not too incompatible. Rather, the states form two poles of a continuum (a realistic assumption for the plant trade, where premises and plant shipments can have a varying proportion of infected plants).

In 2009, the United States imported 1.2 billion live plants, 2.6 million pounds of seed for planting, and countless shipments of horticultural plants that pass through inspection and enter the retail distribution system in less time than the latent period for most diseases. The tremendous volume of trade of plants and plant products crossing borders and ecological areas and the speed of that trade precludes inspection and interception as a primary strategy for preventing the emergence of plant pathogens (Stack, 2010; Stack et al., 2010). On average, 1–2 percent of U.S. imports are inspected. Even with effective surveillance and diagnostic systems, the emergence of fungal plant pathogens will continue. Existing natural dispersal pathways at the site of introduction, in addition to local and regional trade channels, provide opportunities for spread from the sites of introduction.

The first comprehensive inventory of alien fungi and oomycetes recorded in France since 1800 was recently reported (Desprez-Loustau et al., 2010), with some 65 percent being plant pathogens. Using this dataset, the factors influencing invasion success were investigated, with an emphasis on forest tree pathogens. There was an influence of climatic factors, but human population size and its relationship with imports of plants in trade was a major explanatory variable.

In some cases, it is not simply the occurrence of a plant health problem in trade that is the issue, but that of human or animal health, notably with regard to mycotoxins. For example, the occurrence of tracheomycosis associated with *Gibberella xylarioides* on Robusta coffee in East Africa (Hindorf, 1998) has added to the long-known mycotoxin problem in raw coffee, limiting imports, especially to the European Union. Similarly, the increasing trade volumes of fresh produce due to consumer appreciation of the health benefits brought by consumption of fresh fruit and vegetables are associated with a greater occurrence of outbreaks of foodborne human pathogens, such as *Salmonellae*, *Escherichia coli*, noroviruses, and hepatitis A (Berger et al., 2010).

Dispersal of Plant Pathogens as a Cause and Consequence of Warfare and Social Unrest

Perhaps it is no coincidence that sometimes new pathogen problems emerge during periods of warfare and social upheaval. The Bengal famine in the mid 1940s due to *Cochliobolus miyabeanus* was associated with severe weather, but it also occurred at the end of World War II, when many military, political, and social issues constrained the ability to deal with the problem: enough food was there, but it was not distributed to those who needed it (Padmanabhan, 1973). In some cases, famines due to fungal pathogens can lead to, rather than be caused by, periods of social change, as shown by the Irish migrations following the famine caused by *Phytophthora infestans* in the middle of the 19th century (Fry and Goodwin, 1997). In central Italy, the invasion of stone pine stands by North American isolates of *Heterobasidion annosum* was linked to the movement of U.S. troops during World War II (Gonthier et al., 2007). Since that time, there has been a rate of advance of 1.3 km/year along invasion corridors, with the North American taxon dominant, but also some hybridization with the European taxon. The two pathogens are active in the Circeo National Park, where they cause extensive (up to 30 m in radius) gaps in *Pinus pinea* plantations (Scirè et al., 2011). Subsequent studies showed that invasion success was due to the reproductive potential of the invader not being reduced during the dry seasons, when compared with the resident (Garbelotto et al., 2010). Unusually, this example of a fungal plant pathogen has been commented on (“Trees become casualties of war”) in a respected medical journal (Dixon, 2005). Although there have been cases of plant pathogen outbreaks during wars and revolutions, today there is a trend away from looking at climate change with a disaster-focused perspective to an emphasis on long-term livelihood security and adaptation (Conway and Schipper, 2011). For example, the shift from today’s widespread reliance on chemical fungicides to the adoption of alternative and less toxic products such as inorganic salts may happen following democratic regulations and in agreement with consumers’ perceptions, rather than as a result of, for example, mass protests or social unrest (Deliopoulos et al., 2010).

The intentional introduction of plant pathogens as a strategy of warfare has long been considered (Madden and Wheelis, 2003). Concern regarding the creation and deployment of novel pathogen genotypes with high virulence and/or expanded host range could certainly result in the emergence of novel fungal plant pathogens. Although there are clear examples of state-sponsored programs from the past, there is considerable speculation about the relative importance of intentional introductions in the face of introductions resulting from trade and natural events (Stack et al., 2010; Young et al., 2008).

Dispersal Associated with the Introduction of Novel Crops and Plant Species

Farmers world-wide tend to maximize the potential of the crops they grow, in ways that are appropriate to their socioeconomic circumstances, but often only sensible in the short term or not considering side effects such as enhanced opportunities for plant pathogens. For example, modern agriculture relies heavily on increasing the size of fields to enable a more efficient mechanization and economies of scale. But this leads to more uniform landscapes, with potential repercussions on epidemic thresholds of plant pathogens (Moslonka-Lefebvre et al., 2011). Not just in natural ecosystems (Scherrer and Körner, 2011), but probably also in agriculture, small-scale topographic variability will be important for plant species to be able to cope with climate change. In some cases, there may be opportunities to grow a novel crop because the changing climate makes production possible in areas not previously suitable. This may lead to hosts for plant pathogens that were previously unavailable.

In other cases, the cultivation of novel crops arises from other, often political inducements: for example, the requirement that each country in the European Union must derive a certain percentage of its energy use from renewable sources. Hence, there has been a diversion of food crop (cereal) production to bioenergy use, and the planting of non-food crops such as willow, and other short-rotation coppiced trees, and grasses such as *Miscanthus* for such use. As pointed out by Stewart and Cromey (2010), new disease threats, often from fungal plant pathogens, are likely to emerge among the existing ones. This is likely to occur because the crop is new or because the cultivation method (e.g., high-density monoculture) is different from previous practice. In conservation biology, there is a hefty discussion on the issue of assisted migration (managed relocation), which is thought to be going to be necessary for the many species not able to track the predicted rapid variations in climate, although it will result in artificial shifts in species distributions and ecosystems (Loss et al., 2011). In such debates, little attention has been paid to the likelihood that assisted migration will result in unwanted introductions of, for example, exotic plant pathogens.

Invasion Biology and Biodiversity

Dispersal and invasion of pathogenic fungi are significant not only for crop and other managed plant populations such as commercial forests and grasslands, but also for native plant communities (Alexander, 2010; Mordecai, 2011). In such plant communities, invasive alien plants have long been considered a threat to native biodiversity. Conversely, we now admit that native fungal diseases are important components of healthy forests (Ostry and Laflamme, 2009). However, invasive plant pathogens (either introduced with alien plant invaders or through transfer from plants in trade) can pose a threat to ecosystems (Busby and Canham, 2011; Evans and Finkral, 2010; Holzmüller et al., 2010; Loo, 2009; Newcombe and Dugan, 2010; Orwig, 2002).

Phytophthora ramorum is one such example. On the West Coast of the United States, the pathogen affects a range of forest tree and shrub species, from bay laurel to redwoods and tanoaks. In the United Kingdom (U.K.), the main host in woodlands that supports sporulation of *Phytophthora ramorum* is *Rhododendrum ponticum*, itself an invasive plant against which eradication efforts have been targeted, whereas the main native tree species that suffers mortality is beech. The heathland environment and its biodiversity, notably *Vaccinium* and heather species, are also at risk (Harwood et al., 2009). More worryingly, the pathogen has now been discovered affecting large areas of commercially planted Japanese larch (*Larix kaempferi*), introduced into the United Kingdom in the 19th century. Japanese larch also supports sporulation of *Phytophthora ramorum*. Consequently, this poses a threat not only to commercial production, but also to neighboring native trees not previously thought to be susceptible, or never tested (Brasier and Webber, 2010). As has happened in California, infection, tree mortality, and disease spread affect, and in turn are impacted by, forest composition and other aspects such as landscape connectivity, proximity to infected plant nurseries, and woodland history (Davis et al., 2010; Xu et al., 2009).

This example supports the hypothesis mentioned in the Introduction that emerging fungal diseases often result from host shift speciation within a particular ecological context (Giraud et al., 2010). The impact on native biodiversity can usefully be seen from this viewpoint. In return, reduced biodiversity frequently increases disease transmission, including in plants, despite the observation that areas of high endemic biodiversity may harbor a greater source pool of pathogens (Keesing et al., 2010). In general, conserving diverse plant communities reduces the incidence of disease (Pautasso et al., 2005; Turnbull et al., 2010). Of course there are two-way interactions between wild and crop plants, as noted earlier in the case of chickpea and native *Cicer*. *Macrophomina phaseolina* affects a broad range of native plant communities and crops in Kansas. Saleh et al. (2010) found that isolates from crops grown in the vicinity of plants in tallgrass prairie could interchange with the more diverse wild isolates with potential dangers arising from novel recombinations.

Irrespective of how an initial introduction occurred—through natural or

human-mediated means—invasion into endemic communities depends very much on ecological circumstances. For example, the origins of fungi in New Zealand are diverse, a few ancient with many more recent arrivals. Some more recent arrivals have evolved into local endemic species, whereas others are maintained through regular transoceanic arrivals (Johnston, 2010). Since 1980 the number of fungal species (saprobic, mycorrhizal, and pathogenic) has doubled. The kinds of fungi present in New Zealand are constantly changing as a consequence of historical changes in plant and animal biota as well as those exotic fungal species that have become naturalized. The fungal biodiversity of New Zealand may appear today as a rather special case in its exotic nature, but could well become the norm for many other countries if current trends in global trade and carbon emissions will carry on unrelented.

The rates of spread and ecological impact of an introduced plant pathogen through a natural plant community depend on the phylogenetic structure of the plant community (Gilbert and Webb, 2007). Although many of the tropical fungal pathogens investigated by Gilbert and Webb (2007) are polyphagous, the likelihood of any one infecting two species decreases with phylogenetic distance. However, using arbitrary cut-offs, such as plant genus or family, may underestimate the risks of local spread. It would be interesting to integrate this phylogenetic approach with the framework provided by the geographic mosaic theory of coevolution, which was tested for mining moths and leaf rust (*Melampsora*) of *Populus tremula* along a latitudinal gradient in Sweden (Albrechtsen et al., 2010). Climate change can be expected to lead to twists in geographic clines of parasite damage, as well as to shifts in the phylogenetic signal of plant host susceptibility, but it is likely that phylogenetic and spatial structure will still be present even in novel communities and climates.

Much of this overview has been concerned with dispersal and invasion of fungal plant pathogens into crop plants or forest trees (Liebhold et al., 1995). Grassland constitutes a large proportion of the Earth's terrestrial ecosystems, with varying levels of human exploitation (Ellis et al., 2010). Grassland ecosystems have been less studied than crops or forests in the context of climate change and plant health. Reductions in grassland plant richness, a measure of plant biodiversity, appear to increase vulnerability to the spread of fungal plant diseases (Knops et al., 1999), with aspects of global change, especially elevated CO₂ and nitrogen addition, increasing the pathogen load, suggesting this is an important mechanism by which global change affects grassland ecosystems.

Concluding Comments

In cropland, forests, and grasslands, experimentation is needed at the landscape level to investigate plant health adaptation approaches to climate change (Holdenrieder et al., 2004). Similar experimentation, if accompanied by subsequent data analysis and dissemination, is also likely to be beneficial for pathway

control and diagnostic systems (Albers et al., 2010; Norton and Taylor, 2010). Monitoring of plant pathosystems and associated organisms under changing conditions is key to refine models, so as to successfully predict further changes (Luedeling et al., 2011). Similarly, review of the burgeoning literature on the subjects of climate change, land use, and plant health is essential and should be funded on a long-term basis (Jeger and Pautasso, 2008; Pautasso et al., 2010a). Analyses of past and current trends and simulations of future plant health scenarios are likely to be more influential and effective if accompanied by stakeholder involvement and interactions with policy experts (Dwyer, 2011; Mills et al., 2011). Climate change and other global change drivers are not the only factors involved in plant disease epidemiology, so it is still important to consider the important role of, for example, improvements in agronomic and silvicultural practices in reducing current disease potential, whether or not the climate is likely to become more conducive to a certain chronology of diseases in the coming decades (Savary et al., 2011). Nonetheless, it is time to start adapting to the challenges to plant health posed by the future climate change scenarios (Juroszek and von Tiedemann, 2011; Legrève and Duveiller, 2010; Olesen et al., 2011; Planinšek et al., 2011).

The emergence of fungal plant pathogens, facilitated by climate change and globalization, will challenge our ability to meet future food demands for a growing population and to achieve sustainable environments that provide the ecosystem services on which societies depend. Policies informed by science and an infrastructure that supports plant health are prerequisites to that future.

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A15

EMERGING FUNGAL DISEASES OF WILD ANIMAL SPECIES

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Summary

Several fungal diseases of non-domestic animal species have been described as agents of epizootic proportions in wild animals in recent years. The emergence of these diseases has reshaped the understanding of the role of fungi as contagious diseases having an impact on wild animal populations. The recent description of fungal epizootics caused by *Batrachochytrium dendrobatidis* (Bd) in amphibians worldwide and *Geomyces destructans* in North American bats has called attention to the factors driving the emergence of these diseases. Two other fungal diseases of wild animals, lobomycosis in dolphins and penicilliosis in wild bamboo rats, have been recognized for their zoonotic potential and highlight the need for comprehensive, multidisciplinary approaches to understanding the ecology and epidemiology of these diseases in wild animal populations. Continued efforts aimed at mitigating the effects of fungal epizootics on wildlife populations and on

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public health will only be successful through the identification of factors driving the emergence of these diseases across different taxa, and embracing the concept that wildlife may serve as sentinels of ecosystem health.

Introduction

Anthropogenic activities have been the likely driving factors behind the emergence of some diseases in wildlife (Daszak et al., 2000). In some cases, animal species whose status may have been threatened by other factors may now be faced with extinction as the emergent disease spreads through a diminished population. The recent spread of two major fungal epizootic agents, namely *Batrachochytrium dendrobatidis* and *Geomyces destructans* in amphibians and bats, respectively, could result in the largest changes to vertebrate populations in recorded history (Frick et al., 2010; Skerratt et al., 2007). The discovery of these fungal agents as major causes of wildlife epizootics in the past decade has revolutionized the way that biologists approach the detection and diagnosis of fungal diseases, challenging the prior misconception that fungal infections only occurred “sporadically or in small outbreaks” and were more important to captive wildlife, where captivity was thought to “increase susceptibility to these diseases” (Burek, 2001).

As novel pathogens have been discovered, or known ones recognized in novel hosts, in novel geographic areas, or with increased incidence, wildlife conservationists have been faced with the emergence of fungal diseases that threaten the status of wild animal populations. The factors driving disease in free-ranging wild animal species can no longer be easily differentiated from those affecting captive wildlife. The different factors have been blurred into a continuum of factors through modern globalization, inadvertent movement of disease, disease vectors, or animals themselves, through the trade of animals across geographical barriers and the mixing of potential hosts of disease that may not have been exposed to each other in their native habitats. Many of these anthropogenic actions have expanded the geographic range of some diseases or removed the natural barriers that had prevented their spread, exposing naïve hosts to pathogens to which they were not previously exposed. Concurrently, many of the environmental factors thought to predispose their captive counterparts to infectious diseases have been identified and minimized through modern captive animal science aimed at reducing stress levels, providing better environmental conditions, and through better quarantine, improving disease screening and recognition procedures.

Institutions dedicated to the captive care of animals for conservation purposes often encounter infectious diseases that would otherwise go unreported in those species. These provide a useful baseline of understanding the host–pathogen relationship systems. Captive animal populations can serve as viable models for recognizing or refining the understanding of disease mechanisms in individual hosts, which may not be possible in free-ranging animals. For in-

stance, in the 1990s, investigations by pathologists at the Smithsonian's National Zoo of an apparently novel fungus affecting captive frogs led to the isolation of *Batrachochytrium dendrobatidis* (Pessier et al., 1999) and fulfillment of Koch's postulates (Nichols et al., 2001) in what would subsequently be recognized as the most important infectious disease affecting wild amphibian populations on a global scale.

The widespread recognition of other fungal pathogens and their prevalence in wildlife populations has also changed the understanding of their role as agents of contagious potential and concern to human public health. Concern to human health from fungal diseases carried or propagated by wild animals has brought attention to the role of wildlife and interactions with humans in changing environments as indicators of global health and ecosystem stability. Direct zoonotic potential or a shared susceptibility to disease where wildlife have a high prevalence of infection is worthy of direct public concern, but fungal pathogens where the infection potential is limited to wild animals still carry an inherent cost to the health of an ecosystem and carry indirect impacts to human society that may be difficult to quantify.

This review article summarizes some of the fungal diseases currently known to affect wild animal species that could be encountered by veterinarians, conservationists, or animal professionals. This review is not intended as a comprehensive reference on the subject. Instead, it is meant to be a common point for calling attention to the impact of the recent emergence of specific fungal pathogens in wildlife species within the context of wildlife as sentinel species for recognizing changes to the health of an ecosystem.

Emergent Fungal Pathogens of Wild Animal Species

Batrachochytrium dendrobatidis

Batrachochytrium dendrobatidis is a member of a basal group of fungi, the Chytridiomycota, and the only member known to affect vertebrates. *Bd* infects amphibian species, with frogs most often reported. This emerging pathogen was discovered in the late 1990s (Berger et al., 1998; Longcore et al., 1999; Pessier et al., 1999) and has been recognized as a significant pathogen partially implicated in the global decline of amphibian populations (Schloegel et al., 2006; Skerratt et al., 2007). It has been retrospectively identified in North American amphibians as early as 1961 (Ouellet et al., 2005). *Bd* may be the most significant fungal infectious disease agent of vertebrate species, based on the global distribution, wide species host range, pathogenic potential, and ability to cause large-scale mortality.

Molecular genetic evidence from isolates collected from different locations worldwide suggests that recent *Bd* outbreaks were caused by a pathogen recently disseminated (Morehouse et al., 2003), and anthropogenic spread is suspected

in at least some introductions (Weldon et al., 2004), but the origin is still not conclusively known. Virulence and pathogenic potential varies among isolates, and some may show little or no pathogenicity to their hosts (Berger et al., 2005; Goka et al., 2009), but these host-adapted strains may still serve as reservoirs of strains that are pathogenic to other species. The naturally occurring bacterial flora of the host may produce antifungal proteins that decrease the ability of the fungi to colonize amphibian skin, and these bacteria may improve host survival (Harris et al., 2006). Mortality in affected amphibians is likely associated with physiologic abnormalities (electrolyte loss and osmotic imbalances) caused by damage to the permeable amphibian skin.

Clinical signs in affected frogs can be subtle and not detectable before sudden death (Pessier et al., 1999). Affected frogs show excessive shedding of skin, usually on the legs, feet, and ventrum (Nichols et al., 2001). Molecular techniques have been established as a cost-effective and rapid method to detect the organism in amphibian samples (Boyle et al., 2004; Kriger et al., 2006). Histopathology, showing intralesional organisms in keratinized layers of skin, and cytology of shed skin or imprints showing chytrid organisms have been used for diagnosing infections (Nichols et al., 2001). *Bd* has no known zoonotic potential.

Chrysosporium Anamorph of *Nanniziopsis vriesii* (CANV)

Nanniziopsis vriesii is an ascomycetous fungus recognized as a pathogen from skin lesions of reptiles, specifically lizards, snakes, and crocodilians. When cultured, *Nanniziopsis vriesii* produces an anamorph form that is described in the genus *Chrysosporium* and is undistinguishable from reptilian isolates (Paré and Sigler, 2006). Mycotic infections may be morphologically misidentified as other species of *Chrysosporium*, *Geotrichum*, or even *Trichophyton*. Historical reports in the literature of reptilian mycoses attributed to unspecialized members of these genera should be interpreted with caution, as CANV infections may have been previously underdiagnosed. CANV deserves special attention as an emerging fungal pathogen of reptiles because clinical disease has been recognized in a multitude of species with increasing frequency (Abarca et al., 2008; Bertelsen et al., 2005; Bowman et al., 2007; Nichols et al., 1999; Paré and Sigler, 2006; Paré et al., 2006; Thomas et al., 2002), but most reports involve captive or farmed reptiles. Unlike opportunistic fungal pathogens, which are often ubiquitous in the host's environment, the CANV is extremely rare on healthy reptile integument (Paré et al., 2003), suggesting that this organism is a primary fungal pathogen in reptiles.

Preliminary molecular genetic evidence has suggested that the CANV represents a species complex with distinct host affinities (Paré and Sigler, 2006). Until further molecular work is completed, the magnitude of the CANV as a pathogen of free-ranging and captive reptile populations cannot be properly described,

but its potential as an emerging primary fungal pathogen of reptiles cannot be ignored.

Clinical signs vary by species, but when seen in inland bearded dragons (*Pogona vitticeps*), it causes a yellow skin discoloration dubbed “yellow skin disease” or “yellow fungus disease.” The disease can have a protracted, chronic course involving deep tissues (including muscles and bones) and cause disfigurement in addition to the necrotic skin lesions seen. The corresponding lesions can be diagnosed as CANV through histopathology of lesions and fungal cultures. Histopathology typically shows severe granulomatous, necrotic dermatomycosis with the characteristic intralesional fungal organisms. The CANV forms solitary, single-celled conidia that can be confused with the microconidia of dermatophytes (e.g., *Trichophyton*), and arthroconidia caused by fragmented hyphae, which may resemble the arthroconidia of *Geotrichum* or other *Chrysosporium* (Paré and Sigler, 2006).

A single case report of a mycotic brain abscess attributed to the *Chrysosporium* anamorph of *Nanniziopsis vriesii* in an HIV-seropositive Nigerian man has suggested that this reptile pathogen can be a zoonotic agent, although the route or source of exposure or infection in this patient could not be identified (Steininger et al., 2005).

Geomyces destructans

Geomyces destructans is a newly described psychrophilic fungus that affects North American bats during hibernation (Gargas et al., 2009), causing the characteristic fungal white growth after which the syndrome was originally named.

White-nose syndrome (WNS) could more accurately be dubbed bat geomycosis (per Chaturvedi and Chaturvedi, 2011), but the denomination of WNS has been used since its recognition (Blehert et al., 2009). Its impact on wild bat populations has earned it the distinction of being the second most significant vertebrate pathogen in recorded history (after *Bd*), if only because *Bd* has been documented in more species, with a wider global impact.

Bat geomycosis is a true emerging disease of epizootic proportion, appearing in upstate New York in 2006 (Blehert et al., 2009), but quickly spreading south and westward and devastating bat populations (Blehert et al., 2009; Frick et al., 2010). Host susceptibility to infection seems to vary with species. The fungus has also been identified in Europe (Puechmaille et al., 2010; Wibbelt et al., 2010), but to date, the severe, widespread mortality seen in North American bats has not been documented. Among North American bat species, either pathologic lesions or *G. destructans* DNA has been detected in the gray bat (*Myotis grisescens*), the Indiana bat (*M. sodalis*), the little brown bat (*Myotis lucifugus*), the northern long-eared bat (*M. septentrionalis*), the eastern small-footed bat (*M. leibii*), the southeastern bat (*M. austroriparius*), the cave bat (*M. velifer*), the tricolored bat

(*Perimyotis subflavus*), and the big brown bat (*Eptesicus fuscus*) (Foley et al., 2011).

Geomyces destructans infections in bats result in premature arousal from hibernation and abnormal behavior. Although the mechanisms by which death occurs are still under investigation, the inability to forage in the winter after premature arousal (due to lack of prey availability) and direct damage to the wing membranes, resulting in irreversible physiologic and homeostatic deficits from which bats cannot recover (Cryan et al., 2010), are significant factors.

Limiting human access to caves of concern has been a management tool implemented to limit the spread of the *Geomyces* fungus. One of the first theories to explain the rapid appearance and spread of this pathogen in North American bat populations suggested that the fungus may have been endemically established in European bat populations and that a recent anthropogenic introduction into North America may have led to disease in naïve populations, but this theory has not been tested. Histopathology of rostral muzzle and wing membranes is deemed important to confirm infections and establish true prevalence of this disease (Meteyer et al., 2009), although advanced diagnostic tools are likely being developed and refined.

As bats are significant providers of ecological services, such as insect control and pollination, the extinction or even reduction of bat populations is likely to have economic impacts on society beyond the immediate loss of biodiversity.

Penicillium marneffe

Penicillium marneffe is the only fungus of this genus known to be a primary pathogen of mammals. *Penicilliosis marneffe* is a systemic fungal disease of wild rodents and humans recognized in northeast India and Southeast Asia (Thailand, the Guangxi region of China, Vietnam, Taiwan, and Hong Kong). *Penicillium marneffe* was first identified from hepatic lesions in a bamboo rat (*Rhizomys sinensis*) from Dalat, South Vietnam (Caponi et al., 1956), and was subsequently identified as a human pathogen following an accidental exposure by a researcher (Vanittanakom et al., 2006). It is an emerging human disease, a primary pathogen to bamboo rats, and a threat to public health. *Penicilliosis marneffe* is third only to tuberculosis and cryptococcosis as the most common opportunistic infections in patients with AIDS in northern Thailand (Vanittanakom et al., 2006), and the source of more than 100 cases per year in the Guangxi region of China (Cao et al., 2011).

Affected rodents show ascites and enlargement of the liver, spleen, and lymph nodes. The fungal pathogens can be identified in multiple organs and ascitic fluid, but is most commonly cultured from the lung of affected rodents (Ajello et al., 1995).

Wild bamboo rats may be good sentinels of this sapronotic disease, and are significant in the ecology of this disease, although their exact role is not com-

pletely understood. *Penicillium marneffei* has been isolated from the internal organs of four species of bamboo rats (*Rhizomys sinensis*, *Rhizomys pruinosus*, *Rhizomys sumatrensis*, and *Cannomys badius*) and from the soil associated with their burrows (Vanittanakom et al., 2006). A high prevalence of infection and lesions among wild bamboo rats of the genera *Rhizomys* and *Cannomys* suggested that these wild animals may serve as enzootic reservoirs, but the prevalence varies across regions, and a wildlife reservoir has never been conclusively identified (Cao et al., 2011; Deng et al., 1986; Gugnani et al., 2004; Vanittanakom et al., 2006). When sympatric rodents have been sampled, only bamboo rats appear to harbor the organism (Gugnani et al., 2004), suggesting distinct host differences.

Possibly, bamboo rats are exposed from either an unidentified wildlife vector or an environmental source (Vanittanakom et al., 2006), and are not the wildlife hosts of this disease, although they can transmit the disease to humans. The role of wild bamboo rats as amplifiers or dispersers of infectious stages has not been eliminated. The initial case of human infection in 1959 in a researcher who became infected after injection from a needle used in laboratory inoculations (Vanittanakom et al., 2006) underscores the zoonotic risk posed by wildlife.

Although most human cases involve immunosuppressed individuals, infections in humans with normal immunity—and the lack of evidence for immunosuppression in affected bamboo rats—suggests that *P. marneffei* could be a primary mammalian fungal pathogen (Duong, 1996). A complete understanding of the role of wildlife in this disease and its dynamics in rodent populations is essential to better managing the threat to human health in endemic regions and mitigating the possible anthropogenic spread of this disease through travel, movement of commercial products (some of which may be environmental reservoirs), and the primary trade of wild animals. *Penicilliosis marneffei* remains one of the most enigmatic emerging fungal diseases of wildlife, and one whose primary zoonotic potential is not fully understood.

Lacazia loboi

Lobomycosis is a zoonotic disease of dolphins caused by *Lacazia loboi*, a cutaneous fungus in the order Onygenales that has never been cultured (Herr et al., 2001). The disease affects humans and dolphins in tropical and transitional tropical climates. Lobomycosis is endemic in certain human populations in Central and South America, and is likely endemic in regional dolphin populations (Murdoch et al., 2008), although the overall prevalence and many factors of its ecology are still unclear. The disease has been confirmed in two dolphin species, Guiana dolphins (*Sotalia guianensis*) (Van Bresse et al., 2009) and Atlantic bottlenose dolphins (*Tursiops truncatus*) (Caldwell et al., 1975), and has been suspected in Indo-Pacific bottlenose dolphins (*T. aduncus*) (Kiszka et al., 2009).

Transmission between dolphins is likely by direct contact, and affected dolphins show chronic white to pink verrucous, raised lesions that may coalesce

into large plaques or nodules predominantly on dorsal and pectoral fins, the head, fluke, and caudal peduncle (Murdoch et al., 2010). Lesions may be associated with sites of prior trauma, and many affected dolphins have shown impaired adaptive immunity, suggesting that the disease may represent an opportunistic infection in an immunocompromised host (Reif et al., 2008).

Some have suggested that the disease in dolphins is being more frequently reported (Van Bressem et al., 2009) and the reported geographic range may be expanding (Rotstein et al., 2009), making it a true emerging fungal disease of wildlife. In the Indian River lagoon of Florida, measured prevalence was 30 percent in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the southern part of the lagoon in a 2006 survey (Reif et al., 2006). It was not detected in the northern portion (near Charleston, South Carolina), suggesting that localized geographical factors or environmental stressors may play a significant role in the incidence and distribution of the disease. A subsequent study (Murdoch et al., 2008) suggested that the disease is endemic and not an emerging disease in the Indian River dolphin population. A survey of Guiana dolphins (Van Bressem et al., 2009) in the Paranaguá estuary in Brazil suggested an increasing detection of a lobomycosis-like disease (missing histological confirmation) and suggested that the change was an indication of the health of the marine environment.

Coccidioides immitis and *Coccidioides posadasii*

Coccidioidomycosis is a systemic fungal disease known to affect a large number of domestic and non-domestic animal species, both in captivity and in the wild. It is a primary pathogen and can affect otherwise healthy individuals, although clinical disease is often seen in animals with recognized limited immune function or underlying disease. *Coccidioides immitis* is considered endemic to the Western Hemisphere, and is commonly recognized in the southwestern United States in the Sonoran life zone—areas characterized by alkaline soils and an arid climate. Coccidioidomycosis is also reported in Mexico and Central and South America, although *C. posadasii* is often implicated in infections outside of the Southwestern United States. Anthropogenic disturbance of soil (e.g., construction), dust storms, and cycles of drought may precede outbreaks, but wind-blown spores have been hypothesized to play a role in cases of affected marine mammals far from endemic areas. Fungal spores may be concentrated around rodent burrows, but wild animals are not likely reservoirs of this disease and contagion is unlikely to occur between animals. Human exposure may occur during necropsies of affected carcasses by aerosolization of spores, but wild animals are likely insignificant in the epidemiology of the disease in wild animal populations.

Coccidioides has affected some captive endangered species disproportionately more than other species, and can be a threat to species with limited immune function. Specifically, the Przewalski's horse (*Equus przewalskii*) has been disproportionately affected in areas where the disease may be endemic (Terio et al.,

2003). In one area of southern California, coccidioidomycosis was associated with high incidence of infection and was the leading cause of death in a population of this endangered species, posing a threat to the captive propagation of this species. The disease showed a predilection toward younger male horses, and it has been suggested that the immune system of some of these horses may not respond appropriately to infection with coccidioides (Terio et al., 2003). Limited immune function can be a limitation of propagation of endangered species with a limited genetic pool, and infectious diseases can have disproportionate effects on these populations, even when the epizootic potential is limited.

Actions for Managing and Mitigating the Effects of Fungal Diseases on Wild Animals

A basic understanding of the role of fungal diseases in populations is the most important, fundamental need to manage the spread of these diseases. Public expectation for action in the face of spreading epizootics is high, and basic knowledge gaps must be addressed to develop and implement effective management plans. Although the goal is to correct the product of detrimental anthropogenic influences on the occurrence or emergence of diseases, intentions and action plans should be tempered by the recognition that naturally occurring diseases are and have been a driving evolutionary and ecological force responsible for shaping and adapting community structures in wild populations. Adaptive management strategies should be scientifically sound.

In the absence of proven action plans for the control of specific fungal pathogens, general action plans for the adaptive management of fungal epizootics can be modeled following the template considered for the control of WNS in bats (Foley et al., 2011). These strategies can generally include: continuing disease and population surveillance; limiting further anthropogenic spread through biosecurity and quarantine measures; providing individual animal treatments to increase survival; creating protected populations or rescue populations with the goal of preserving genetic diversity; increasing population resistance to the disease; modifying the environment or environmental sources of exposure; selective culling of populations to limit transmission; and disseminating factual information to address both public perception and foster scientific collaboration and research.

Prospective monitoring and surveillance of disease and populations allows wildlife managers to establish objective criteria that can be used to document their impact following the emergence of a pathogen. In the case of a novel pathogen, such as the *Geomyces destructans* in North American bats, historical, basic data on the population status of native bat populations has allowed the documentation of changes to the population after the emergence of this disease. By contrast, the lack of historical specimens collected at the time of initial decline of amphibian populations, whose decline has been attributed to chytridiomycosis, has raised unanswerable questions about the cofactors that may have precipitated the

emergence of this fungal pathogen or its true impact on populations (McCallum, 2005). Active surveillance should rely on standardized case definitions, sampling methodologies, and data analysis and centralized sharing of results. Molecular techniques, some of which were in their infancy a mere 10 years ago, have allowed the differentiation of pathogenic strains for different fungal organisms and their relationship to the host and their immune defenses, and a better understanding of the ecology of fungal diseases. For instance, the advancement of molecular techniques as an adjunct to field surveillance techniques has proven valuable in understanding the dynamics of amphibian chytridiomycosis. The characterization and dissemination of reports of fungal diseases in novel hosts or new presentations in known hosts is an implied responsibility of veterinarians and animal biologists working with wildlife species, both in captive or free-ranging situations.

In the face of spreading epizootics, movement and entry restrictions or quarantine regulations are often imposed to halt or slow down the spread of an infectious disease. These restrictions can be as simple as prohibiting (human) visitor access to caves where bat geomycosis has been documented, or as complex as trade regulations and sanctions against the commercial movement of species. Instituting proper quarantine procedures when entering endemic areas and educating visitors and researchers about their own potential as vectors of disease can be effective methods to avoid irresponsible spread of pathogens. Responsible disinfection protocols can be established by scientists repeatedly monitoring affected populations.

Individual animal treatments may not be feasible or effective in halting the large-scale spread of epizootics, but are valuable if the animals treated are being used for captive propagation programs or if treatment is only needed for extremely small populations. Treatment of infected individuals may include antifungal agents, although the efficacy and safety of these for individual fungal pathogens has not always been established, and a specific discussion of antifungal agents and their efficacy in the treatment of specific pathogens is beyond the scope of this manuscript. The biggest limitations in delivering treatments to free-ranging wild animals is lack of access to treat individual animals, and lack of knowledge on the proportion of a population that would need to be effectively treated to reduce the continued spread of a fungal disease. Large-scale aerosolization (“fogging”) treatments have been suggested for specific cave environments affected by bat geomycosis, but the environmental effects of “untargeted” environmental treatments are likely to disrupt microbial communities within delicate cave ecosystems, and the efficacy for the treatment is unknown at this time.

The captive breeding of endangered species vulnerable to emerging infectious disease pandemics has been advocated as a temporary “rescue” measure until the threat of infectious disease can be mitigated. Captive breeding is particularly appealing as a putative solution when the infectious disease has had an anthropogenic component, or when human activities have compounded a threat to vulnerable populations. In principle, captive stock can be subsequently used to

supplement wildlife populations or reintroduce them, after the infectious disease threat is mitigated, to areas where the species underwent local extinction. Indeed, many species have been successfully bred in captivity for purposes of reintroduction after the initial threat of an infectious disease has threatened their survival.

A successful captive breeding and propagation program undertaken by the U.S. Fish and Wildlife Service (FWS) in the 1980s was aimed at the recovery of the black-footed ferret (*Mustela nigripes*). Although the species was considered extinct in 1979, the rediscovery of a small population in Wyoming in 1981 launched a recovery program for the species. When this extremely small population was further threatened by an outbreak of canine distemper virus after a sylvatic plague outbreak (Forrest et al., 1988; Williams et al., 1988), the FWS started a rescue effort to propagate the species in captivity from those wild founders. This program has now resulted in the reintroduction of the species into the wild at multiple sites, and has resulted in adequate early success in a relatively short time frame for the recovery of an entire species. Between 1987 and 2010, more than 6,500 ferret kits were produced, and over 2,300 animals have been released into the wild through 19 projects across 8 states, Canada, and Mexico (FWS, 2011).

Over the years, this program has benefited from advances in veterinary medicine to mitigate the threats posed by infectious diseases in the wild and in captivity; advances in captive husbandry and nutrition; establishment of biosecurity measures; application of modern principles of genetic management; and the use of assisted reproductive techniques to enhance genetic diversity. Similar multidisciplinary programs could be applicable to supplement endangered populations at risk of extinction from a fungal disease.

The unprecedented emergence of amphibian chytridiomycosis and the global scale of this threat have triggered the launch of concerted recovery efforts for amphibians under the Amphibian Ark (AARK). The AARK is a joint initiative by international partners (the World Association of Zoos and Aquariums, the International Union for Conservation of Nature [IUCN] Conservation Breeding Specialist Group, and the IUCN Amphibian Specialist Group) dedicated to “ensuring the global survival of amphibians, focusing on those that cannot currently be safeguarded in nature” (AARK, 2011). The AARK prioritizes and rescues amphibian species at risk of extinction while developing in-country capacity for the continued research and propagation of those species. Although aimed at the overall decline of amphibian populations, the prominent role of the chytrid fungus in the decline has prompted the AARK to develop and refine biosecurity procedures and management techniques to control and mitigate the effects of this disease. This active network of professionals is a model for the recovery of species through ex situ propagation and investigation, with the goal of returning and recovering threatened populations of amphibians to their natural habitats.

However, captive propagation may not always be the most viable solution in the face of a wildlife epizootic, and should never be seen as a substitute to directly addressing the anthropogenic factors driving the emergence of diseases threaten-

ing wildlife populations. The financial cost of captive propagation programs is variable and species-dependent, but inevitably significant. In addition, the life history and biological needs of some species may make them unsuitable candidates for captive recovery programs. Captive breeding should be seen as a short-term strategy for the recovery of endangered or threatened species, and should only be considered as a last resort, as in the previous examples, with defined goals and a target time line. Captive breeding should only be undertaken in conjunction with comprehensive efforts to create in situ capacity for disease and population monitoring, continued basic disease research, and efforts to contain or mitigate the spread of disease in the wild. The limitations of captive breeding programs have been previously discussed (Lynch and O'Hely, 2001; Snyder et al., 1996) and should always be weighed as part of the risk assessment on the viability of captive breeding strategies. Limitations include problems with establishing self-sufficient captive populations; species-specific and possibly limited success in reintroductions; relatively high costs; possible domestication and the selection and propagation of traits that may not be ideal for wild species survival; and possible exposure and establishment of unrecognized diseases in captive stock that could be carried to their wild counterparts.

Techniques that increase the population resistance to infection could be applicable to the management of fungal epizootics, specifically through vaccinations or bioaugmentation of biological defenses. The acquisition of immunity against fungal pathogens is not well known for all pathogens, and the complexity of pathogen–host relationships further exacerbates our gaps in knowledge. Although not widely used, fungal vaccines have been developed, even if their efficacy has varied widely. Different strategies in vaccine development, from targeting fungal cell wall characteristics (Cassone and Torosantucci, 2006) to developing recombinant vaccines that induce immunity to specific fungal proteins, could be employed. However, these techniques take time to develop and effectively implement on a large scale, and are potentially costly.

A novel technique being used to convey immunity to amphibians at risk of chytridiomycosis involves the use of skin bacteria that produce antifungal compounds. The addition of *Janthobacterium lividum* to the skin of mountain yellow-legged frogs (*Rana muscosa*) has been demonstrated to convey protection against challenge with *B. dermatitis* (Harris et al., 2009). Surveillance of wild populations has suggested that the presence of symbiotic skin bacteria capable of producing anti-*Bd* compounds is beneficial to populations in the wild (Lam et al., 2010). The bioaugmentation of anti-*Bd* bacterial flora has the potential to be a significant advance in increasing the survival of amphibians in areas where the fungus has become endemic. The recognition of similar symbionts in the skin of other species could help direct action plans toward increasing resistance to fungi in natural populations.

Environmental manipulations could be effective in captive efforts, or during short-term recovery of individual infected animals, but are not necessarily

feasible to apply in wild environments. Manipulating the pH of soil or water, and changing the temperature, humidity, or other microclimatic conditions, are possible ways to discourage the growth of certain life stages of fungal organisms. However, basic biological research is necessary before these manipulations could even be considered or applied in the wild. The detrimental effects of these manipulations to the environment would need to be understood and minimized, particularly when applied at scales large enough to make a difference to the health of a population of wild animals.

The mass culling of populations known to be affected by an emerging infectious disease has been advocated by domestic animal regulatory agencies and has been an effective tool in the eradication of diseases of agricultural interest and zoonotic concern. The efficacy of culling wildlife populations has not been established and efforts have been met with mixed results. Success would depend on a multitude of factors, including the pathogen, transmission dynamics, population biology of hosts and the reservoir species, prevalence and incidence of the disease, and culling techniques. Wildlife culling has been effective at meeting the immediate management goals in some wildlife health scenarios, but the overall impacts to ecosystems are often unknown, typically complex, and not easily predicted. For instance, long-term efforts to cull wild European badgers (*Meles meles*) by the British government to control tuberculosis (TB) in cattle have been successful at reducing incidence of TB in cattle in the immediate area where badgers are culled, but actually increased the incidence in adjoining areas (Donnelly et al., 2007). A disease model to examine the efficacy of culling bats in hibernacula as a way to slow the spread of WNS suggested that this strategy would be ineffective, in part due to high contact rates among colonial bat species (Hallam and McCracken, 2011). Culling is not likely to be a large-scale effective strategy for managing fungal infectious diseases.

Conclusion

Several emerging fungal diseases have recently been shown to affect wild animal species. Some of these diseases carry a zoonotic risk potential, but even those that do not directly affect human health are likely to carry a societal cost in terms of ecosystem health. Wild animal species can be sentinels of emerging diseases and are early indicators of overall ecosystem health. Advances in veterinary medicine have aided in the recognition of fungal pathogens, and new techniques are being developed to mitigate their impact on wild populations. However, the inherent responsibility falls to veterinarians, physicians, conservation biologists, and public health professionals to properly document and disseminate the findings of fungal diseases in novel hosts, geographic locations, or areas of increased frequency. Continued multidisciplinary surveillance of fungal diseases is essential to understand the impact of these emerging pathogens on wild animal populations, humans, and ecosystems.

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A16

THE EMERGENCE OF *PHYTOPHTHORA RAMORUM* IN NORTH AMERICA AND EUROPE

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Introduction

The emergence of fungal and fungal-like plant pathogens in agricultural and natural ecosystems can be triggered by a number of key changes in the host–pathogen–environment interaction (Anderson et al., 2004; Desprez-Loustau and Rizzo, 2011; Desprez-Loustau et al., 2007). The evolution of pathogens owing to selection pressure due to fungicides, deployment of resistant plant varieties, or changes in the environment (global to local) can allow previously known pathogens to increase in incidence locally or across wide geographic areas (Anderson et al., 2004). Movement of fungi from one part of the world to another may also lead to disease emergence and large-scale epidemics of plant pathogens (Desprez-Loustau et al., 2007; Rizzo, 2005). Because of the well-known impacts of exotic plant pathogens, much effort has been made at regional, national, and

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international levels to restrict movement of plant pathogens in order to protect agricultural crops. However, some of the greatest impacts of exotic plant pathogens have occurred in natural ecosystems. Well-known, high-impact fungal diseases that are considered to be caused by exotic pathogens include chestnut blight (caused by *Cryphonectria parasitica*), white pine blister rust (caused by *Cronartium ribicola*), Dutch elm disease (caused by *Ophiostoma ulmi* and *O. novo-ulmi*), and jarrah dieback (caused by *Phytophthora cinnamomi*) (Aukema et al., 2010; Desprez-Loustau et al., 2007; Rizzo, 2005).

Recently, *Phytophthora ramorum* has emerged as a presumed exotic causal agent of the forest disease “sudden oak death” that has had important impacts on coastal oak forests in California and Oregon (Rizzo and Garbelotto, 2003) and more recently in woodlands in the United Kingdom (Brasier and Webber, 2010). There are several reviews available that cover disease symptoms, biology, ecology, management, and history of *P. ramorum* (e.g., Davidson et al., 2003; Garbelotto and Rizzo, 2005; Grünwald et al., 2008; Kliejunas, 2010; Rizzo and Garbelotto, 2003; Rizzo et al., 2005). Information on the pathogen and the diseases it causes is updated regularly on the website of the California Oak Mortality Task Force (<http://www.suddenoakdeath.org>). In this brief review, we give an overview of some of the factors responsible for the emergence of *P. ramorum* followed by current work on management of the pathogen.

The Pathogen

Phytophthora ramorum was unknown before it was observed to cause diseases of a number of host species in the mid-1990s in Europe and California. The pathogen was officially named *P. ramorum* in 2001 (Werres et al., 2001). Population studies indicate that *P. ramorum* in both North America and Europe has a genetic structure consistent with that expected of an introduced species and that it reproduces exclusively clonally (Ivors et al., 2004, 2006). *P. ramorum* has two mating types (A1, A2); to date, all North American isolates of *P. ramorum* have been found to be mating type A2, while all isolates in Europe (with rare exceptions) are A1 (Grünwald et al., 2008). Sexual reproduction outside of the laboratory has not been documented (Grünwald et al., 2008), although laboratory attempts at crossing the *P. ramorum* mating types have produced viable progeny with similar virulence to the parent types (Boutet et al., 2010). Three genetically distinct lineages (NA1, NA2, and EU1) have been identified within *P. ramorum* (Grünwald et al., 2008; Ivors et al., 2006). Only NA1 is present in California and Oregon forests, all three lineages are found in North American nursery populations, and only EU1 has been found in European nurseries and woodlands (Goss et al., 2009, 2011; Grünwald et al., 2008; Ivors et al., 2006).

The putative exotic nature of *P. ramorum* in North America is also supported by the findings that it is present in nurseries (Yakabe et al., 2009) yet is absent in historical herbarium collections (Monahan et al., 2008). However, the geographic origin of *P. ramorum* remains unknown. From a phylogenetic perspective, its

nearest relative is *P. lateralis*, another presumed exotic pathogen in California and Oregon that is the causal agent of Port-Orford cedar root disease (Rizzo et al., 2002). The geographic origin of *P. lateralis* was also unknown until it was recently found in the mountains of Taiwan (Brasier et al., 2010). This suggests a potential origin for *P. ramorum* in Asia (Brasier et al., 2010), but there is no direct evidence at this time.

P. ramorum is a generalist plant pathogen and has been found to infect more than 125 plant species including ferns, gymnosperms, monocots, and dicots (Grünwald et al., 2008; Kliejunas, 2010). The U.S. Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine maintains an updated list of regulated and associated hosts (<http://www.aphis.usda.gov/>). The diseases that *P. ramorum* causes on this wide range of hosts are expressed in two ways: canker infections that may cause tree mortality and non-lethal foliar and twig infections (known as ramorum blight) (Rizzo et al., 2005). Canker symptoms have been primarily described from oaks and other members of Fagaceae (known as sudden oak death). Recently, canker symptoms have been reported on trunks of Japanese larch in plantations in the United Kingdom (Brasier and Webber, 2010). No host-specific population genetic structure has been detected in *P. ramorum*. While there is variation in virulence within *P. ramorum* populations, isolates of the pathogen from one host show similar virulence on unrelated hosts (Hüberli and Garbelotto, 2011).

Transmission of *P. ramorum* in forests is primarily driven by spore production from foliar infections rather than from lethal cankers on the main stem of trees (Davidson et al., 2005, 2008, 2011). *P. ramorum* spreads naturally via spores over both short and long distances. Spread is primarily by rain splash, with the majority of propagules appearing to travel less than 10 m (Davidson et al., 2005; Mascheretti et al., 2008). Genetic information has been used to infer inoculum dilution curves and spread potential of the pathogen (Mascheretti et al., 2008, 2009). The curve describing such a relationship is bimodal and reminiscent of dispersal curves for relatively large particles, with a steep gradient indicating limited dispersal ability (most dispersal within 10 m, and decreasing to a minimum at about 500 m) and a secondary peak at 1–3 km, indicating medium-distance dispersal (Mascheretti et al., 2008). These values match observations based on the onset of new infestation foci in Oregon (Hansen et al., 2008). Long-distance spread of the pathogen in both North America and Europe appears to be primarily associated with movement of nursery plants (Goss et al., 2009; Ivors et al., 2006; Prospero et al., 2009).

Emergence of *P. ramorum* in North America

A new disease, described as sudden oak death (SOD), was first associated with mortality of tanoak (*Notholithocarpus densiflorus*) and coast live oak (*Quercus agrifolia*) in the San Francisco Bay area during the mid-1990s (Garbelotto et al., 2001). A *Phytophthora* species was identified and confirmed as the causal

agent of the disease in 2000 (Rizzo et al., 2002). In the United States, *P. ramorum*'s current geographic range in native forests extends from the Big Sur area in central California to southern Mendocino County, with two disjunct populations in Humboldt County, California, and one small population in Curry County, Oregon (Figure A16-1). *P. ramorum* has not become established in forests outside of this area. Potentially millions of tanoak and oak trees have been lost to the disease over the past 10 years (Meentemeyer et al., 2008c, 2011).

The pathogen has been associated with the horticultural industry in both the United States and Canada and has been consistently found in a number of nurseries. While dozens of plant species have been found infected in nurseries, the majority of infections have been associated with the genera *Rhododendron*, *Camellia*, *Pieris*, *Kalmia*, and *Viburnum* (Osterbauer et al., 2004; Parke et al., 2010a). Although *P. ramorum* has not been found in forests outside of California and Oregon, the pathogen has become established in streams in several states, including Washington, Alabama, Mississippi, Georgia, and South Carolina (Oak et al., 2010). These streams have been associated with infestations in nearby nurseries; in several rare instances vegetation along the banks has become infected by *P. ramorum*, but there is no evidence of terrestrial colonization of forests at this time outside of California and Oregon (Oak et al., 2010).

The emergence of *P. ramorum* in California and Oregon forests can be linked to several key factors: movement of ornamental plants (both into and within California) (Ivors et al., 2006; Prospero et al., 2009), the susceptibility and distribution of host plants in coastal forests (Anacker et al., 2008; Dodd et al., 2004, 2008; Hayden et al., 2011; Hüberli and Garbelotto, 2011; Meentemeyer et al., 2008a,b,c; Rizzo et al., 2005), and a suitable climate (Meentemeyer et al., 2004, 2011). *P. ramorum* appears to have originally spread from two focal points in California. Two *P. ramorum* populations (Marin and Santa Cruz) were identified using population genetic analysis as the two oldest sources of the pathogen in the state (Mascheretti et al., 2008, 2009). This genetic analysis corroborated anecdotal evidence from field observations and nursery records. By reconstructing the epidemic from this point, other locations were determined to be of intermediate age. For example, the Big Sur population was shown to be derived from the Santa Cruz population originally associated with nursery stock (Mascheretti et al., 2008). This analysis suggests at least eight separate introductions of *P. ramorum* within California from the two focal populations. Most of these introductions were likely human related via movement of nursery plants, but some introductions were likely caused by natural spread of the pathogen from forests (Cushman and Meentemeyer, 2008; Mascheretti et al., 2008, 2009).

P. ramorum can be found in a number of forest types along California and Oregon coasts; these range from drier oak mixed evergreen forests dominated by coast live oak to wetter forest types dominated by coast redwood or Douglas fir (Rizzo and Garbelotto, 2003). In these conifer-dominated forests, tanoak is the

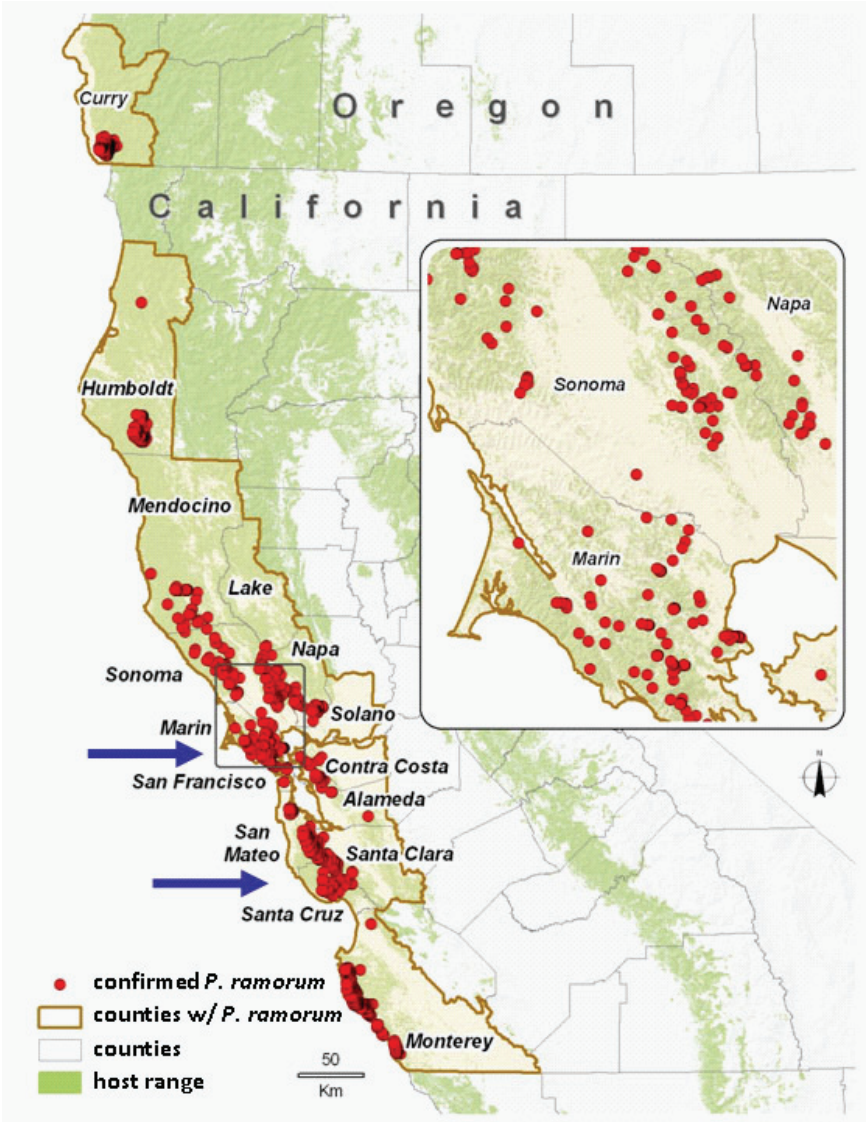


FIGURE A16-1 Current distribution of *Phytophthora ramorum* in California and Oregon forests. Red circles represent areas where *P. ramorum* and sudden oak death have been confirmed. The blue arrows indicate the general locations (Marin and Santa Cruz counties) where *P. ramorum* was initially introduced into California.

SOURCE: Figure courtesy of Ross K. Meentemeyer, University of North Carolina-Charlotte.

host most affected by sudden oak death. As forest types vary across a topographical landscape, there are associated changes in microclimate and, consequently, a likelihood of variation in the host–pathogen interaction and pathogen transmission (Condeso and Meentemeyer, 2007; Meentemeyer et al., 2008a, 2011). These changes in microclimate may be due to variation in edaphic factors such as aspect, soil strata, and hydrology that underlie the growth of a particular vegetation type. Differences in the ensuing physical structure of the vegetation itself also affect light availability and, consequently, temperature and moisture. These are crucial factors that determine survival and sporulation of most pathogens, including *Phytophthora* species. On the level of fine-scale host-pathogen interactions, these environmental variables may affect the timing and production of inoculum, the length of the infectious period, the degree of host susceptibility or pathogen virulence, pathogen survival through adverse conditions (dormancy), the timing and incidence of new infections, and the overall magnitude and pattern of disease epidemics.

Although *P. ramorum* infects more than 25 host species in these woodlands, nonlethal foliar lesions on bay laurel (*Umbellularia californica*) are the most important host tissue for sporulation by *P. ramorum* in the oak woodlands of California (Davidson et al., 2005, 2008, 2011). Levels of inoculum in through-fall rain are up to 20 times higher under bay laurel as opposed to other forest trees. In addition, at the landscape level, infection on bay laurel is known to precede infection on oak and tanoak trees, and the presence of this host is associated with higher levels of oak mortality (Cobb et al., 2010; Maloney et al., 2005). Consequently, infections on bay laurel leaves drive the spread of *P. ramorum* and the onset of lethal infections on oak and tanoak. Sporulation does occur on tanoak leaves and this species does appear capable of driving epidemics in the absence of bay laurel (Davidson et al., 2008). This situation is occurring in Oregon forest, where bay laurel is a relatively minor component of the forests (Hansen et al., 2008).

Various modeling efforts have been made to predict the spread and establishment of *P. ramorum* in California, Oregon, and other locations within North America (e.g., Kelly et al., 2007; Meentemeyer et al., 2004, 2008a,b, 2011; Vaclavik et al., 2010; Venette and Cohen, 2006). For example, a recent epidemiological model was used in combination with geographical modeling to predict the spread of *P. ramorum* through host populations in wildland forests, subject to fluctuating weather conditions (Meentemeyer et al., 2011). Application of the model to Californian landscapes over a 40-year period (1990–2030), since the approximate time of pathogen introduction, suggests that in the absence of extensive control, a 10-fold increase in disease spread will occur between 2010 and 2030 with most infection concentrated along the northern coast of California between San Francisco and Oregon (Meentemeyer et al., 2011). Long-range dispersal of inoculum to susceptible host communities in the Sierra Nevada foothills and coastal southern California leads to little secondary infection due to lower host

availability and less suitable weather conditions. However, a shift to wetter and milder conditions in future years would double the amount of disease spread in California through 2030 (Meentemeyer et al., 2011). In other areas of North America, the forests of the southern Appalachian Mountains are considered to be at the highest risk (Kelly et al., 2007; Rizzo et al., 2005; Venette and Cohen, 2006). The combination of susceptible oaks (e.g., *Q. rubra*), potential sporulating hosts found in the understory (*Rhododendron*, *Kalmia*), and a moist, relatively mild climate has the potential to support the emergence of *P. ramorum* if it is introduced into these areas (Spaulding and Rieske, 2011).

Emergence of *P. ramorum* in Europe

Around the same time that SOD was noted in California, a new *Phytophthora* species was observed to infect rhododendrons in nurseries and gardens in Germany and the Netherlands (Werres et al., 2001). A connection was made between the European and California *Phytophthora* species in December 2000 (Rizzo et al., 2002). Although initially described from Germany and the Netherlands, *P. ramorum* has now been identified in nurseries or gardens of most countries in Western Europe (Kliejunas, 2010). To date, *P. ramorum* has not caused the extensive damage in native European woodlands that has been seen in California forests; stem cankers caused by *P. ramorum* have only been found in the United Kingdom and the Netherlands. The pathogen is most widespread in the United Kingdom (including Ireland) in mixed-species woodlands, planted woodland gardens, heritage gardens, and national plant collections (Fichtner et al., 2011). Surveys for *P. ramorum* in Great Britain were initiated in 2001 and the pathogen has since been found at hundreds of sites, both in nursery systems and in woodlands (Kliejunas, 2010). *P. ramorum* causes extensive foliar necrosis and shoot dieback on *Rhododendron ponticum* (considered an invasive plant species) and bleeding cankers on European beech (*Fagus sylvatica*) as well as other tree species. Early surveys of disease incidence found that the majority of infected trees are located within 2 m of infected *R. ponticum*, suggesting a role of *R. ponticum* in disease transmission.

P. ramorum has recently emerged as a serious pathogen in Japanese larch (*Larix kaempferi*) plantations in the United Kingdom (Brasier and Webber, 2010). Japanese larch is an important timber tree in the United Kingdom and is grown in large plantations. While other conifers had been reported as hosts (e.g., redwood, Douglas fir), this was the first observation of extensive damage caused by *P. ramorum* to conifers. The key finding was that larch can serve both as a canker host, leading to death of large trees (as on oaks), as well as a foliar host that supports sporulation of *P. ramorum* (Brasier and Webber, 2010). The identification of larch as both a foliar and a canker host was unexpected and points to many gaps in the knowledge about the host range and biology of *P. ramorum*.

Disease Management

Management of *P. ramorum*–associated diseases in forests, woodlands, and urban areas has taken a multiscale approach ranging from individual trees to landscapes to international quarantines (Alexander and Lee, 2010; Frankel, 2008; Rizzo et al., 2005). Disease prevention and mitigation at the individual plant level or urban–wildland interface in California has been focused on chemical control or other programs designed to maintain health of plants. Some fungicides have been developed that act as protectants (e.g., phosphonates) against infection, but few chemicals have been developed that work once the plant is infected (Garbelotto and Schmidt, 2009; Garbelotto et al., 2009). Removal of inoculum-producing plants, such as bay laurel or rhododendron, has also been important at smaller scales to protect high-value oaks (Swiecki and Bernhardt, 2008). Education and involvement of local communities has been critical at the urban–wildland interface to the implementation of management programs (Alexander and Lee, 2010).

At larger landscape scales in wildland forest communities, management strategies for *P. ramorum* have included prevention, eradication, treatment, and restoration (Rizzo et al., 2005; Valachovic et al., 2008, 2010). Eradication has been attempted in some cases, most notably with tanoak forests in Oregon (Kanaskie et al., 2010) and larch plantations in the United Kingdom (Brasier and Webber, 2010), but has met with mixed success. Important successes have been balanced by continuing tree mortality in many areas (Kanaskie et al., 2010). Difficulties have been encountered in detecting the pathogen at an early enough stage for eradication to be completely effective at a landscape scale. Cryptic infection (i.e., with minimal or no symptoms) of foliage during the initial invasion of a site by *P. ramorum* has allowed the pathogen to stay one step ahead of detection efforts in many cases. The development of management strategies, beyond eradication, for forest lands following invasion by *P. ramorum* is still in the early stages (Rizzo et al., 2005; Valachovic et al., 2008, 2010). Decision making requires the ability to fit disease management into the context of other management goals (e.g., fire prevention, wildlife) within the broader forest landscape (Rizzo et al., 2005). Examples of approaches that are being tested include forest stand thinning to remove inoculum-producing hosts and use of prescribed fire (Valachovic et al., 2008, 2010).

The broadest scale for disease management, regional to international, is driven by regulations and management practices designed to prevent further spread of *Phytophthora* (Brasier, 2008; Frankel, 2008; Rizzo et al., 2005). In recent years, broadening of national and international quarantines designed to prevent pathogen movement has led to an increased effort to manage all *Phytophthora* diseases in nursery settings (Osterbauer et al., 2004; Parke et al., 2010a,b). While dozens of plant species have been found infected in nurseries, the majority of infections have been associated with the genera *Rhododendron*, *Camellia*, *Pieris*, *Kalmia*, and *Viburnum* (Osterbauer et al., 2004; Parke et al., 2010a). These plant species have become the focal point for development of best management practices and pathogen detection strategies (Parke et al., 2010a). The need for

pathogen detection in nursery plants as part of quarantine inspections has resulted in the development of a number of PCR-based molecular tests (e.g., Hayden et al., 2004; Vettraino et al., 2010).

One consequence of increased detection ability and monitoring for *P. ramorum* has been the discovery of many additional species of *Phytophthora* in nurseries and wildlands (e.g., Brasier, 2008; Hansen et al., 2003; Yakabe et al., 2009). Since 2000, the number of described species of *Phytophthora* has nearly doubled (Brasier, 2009). The ecology and potential long-term impacts of these many “new” species is unknown at this time. Limited knowledge of the baseline biodiversity of *Phytophthora* (and other fungal plant pathogens) in many areas makes it very difficult to determine if an organism is a newly arrived “exotic” species. Therefore, it becomes very difficult to predict if any of these recently described species will have the large ecological impacts of *P. ramorum*.

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A17

CLIMATE CHANGE, EXTREME WEATHER EVENTS, AND FUNGAL DISEASE EMERGENCE AND SPREAD

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Abstract

Empirical evidence from multiple sources shows the Earth has been warming since the late 19th century. More recently, evidence for this warming trend is strongly supported by satellite data since the late 1970s from the cryosphere, atmosphere, oceans, and land. Those data confirm increasing temperature trends and their consequences (e.g., reduced Arctic sea ice, rising sea level, ice sheet mass loss, etc.). At the same time, satellite observations of the Sun show remarkably stable solar cycles since the late 1970s, when direct observations of the Sun's total solar irradiance began. Numerical simulation models, driven in part by assimilated satellite data, suggest that future warming trends will lead to not only a warmer planet, but also a wetter and drier climate, depending on location, in a fashion consistent with large-scale atmospheric processes. Continued global warming poses new opportunities for the emergence and spread of fungal disease, as climate systems change at regional and global scales, and as animal and plant species move into new niches.

Our contribution to this proceedings is organized as follows: First, we review empirical evidence for a warming Earth. Second, we show the Sun is not responsible for the observed warming. Third, we review numerical simulation

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modeling results that project these trends into the future, describing the projected abiotic environment of our planet in the next 40 to 50 years. Fourth, we illustrate how Rift Valley fever outbreaks have been linked to climate, enabling a better understanding of the dynamics of these diseases, and how this has led to the development of an operational predictive outbreak model for this disease in Africa. Fifth, we project how this experience may be applicable to predicting outbreaks of fungal pathogens in a warming world. Last, we describe an example of changing species ranges due to climate change, resulting from recent warming in the Andes and associated glacier melt that has enabled amphibians to colonize higher elevation lakes, only to be followed shortly by the emergence of fungal disease in the new habitats.

Introduction: Observational Evidence for Global Warming

Among many non-scientists, there appears to be controversy over climate change and its causes. This is paradoxical because there is little debate over human-caused climate change within academic communities, except for some peripheral issues, as noted by Lockwood (2009) and others. Let us look to observational evidence from multiple and different sources to better understand climate change and its implications in this persisting pseudo-controversy (Figure A17-1).

A number of methods are used to collect consistent and long-term datasets to monitor Earth's properties. Observations of temperatures by thermometers at the surface and by satellite microwave "sounding" of atmospheric temperature profiles are important components of our understanding of global temperature. Since 2003, we have been able to measure ocean temperature profiles using the ARGO global array of 3,000 free-drifting robotic probes that measure the temperature and salinity of the upper 2,000 meters of the ocean. This provides continuous monitoring of ocean temperature, salinity, and currents, with all data made publicly available within hours after collection (Lyman et al., 2010; Wells et al., 2009). The ARGO data are fundamental to climate studies because the oceans absorb ~90 percent of the heat from global warming.

We can measure the extent of glaciers and their variation over time, frequently drawing on historical paintings, photographs, maps, and satellite image archives to determine if they are getting smaller or larger. We also measure the extent of sea ice weekly and monthly using passive microwave radiometers. More recently, we have been able to measure ice mass variations for the Greenland and Antarctic ice sheets, using gravity data from the joint U.S.–German Gravity Recovery and Climate Experiment (GRACE) satellite mission (Swenson and Wahr, 2002).

We have measured sea level globally since 1993 using radar altimeters on-board satellites. Sea level is an unequivocal proxy for global warming: As the Earth warms, sea level rises; as it cools, sea level falls. Lastly, since 1979 we have been able to measure the Sun's energy output using satellites above the Earth's

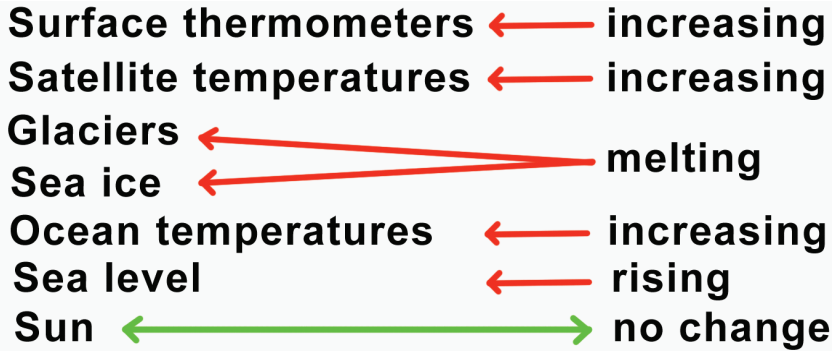


FIGURE A17-1 Summary of observations that show the Earth is warming (red arrows) while the Sun has been constant over the same period of time.

surface. The convergence of observational evidence from all of these sources makes a compelling case that the Earth is warming, and this warming is not due to the Sun.

Convergence of Observations Showing Global Warming

Surface Thermometers

Four global surface temperature datasets are available from the following locations: (1) National Aeronautics and Space Administration (NASA)/Goddard Institute of Space Studies (Hansen et al., 2010), (2) National Oceanic and Atmospheric Administration's National Climatic Data Center (Smith et al., 2008), (3) the University of East Anglia's Climate Research Unit (Rayner et al., 2006), and (4) the Japanese Meteorological Agency.⁵⁴ These four datasets all use the same input data and differ only in interpolation techniques between sparse observations, how the polar regions are treated, and the reference period for which means are calculated. Not surprisingly, they are very similar (Figure A17-2).

Atmospheric Temperature Profiles

Since late 1978, polar-orbiting satellites have provided global air temperature profiles with altitude or "soundings" using passive microwave radiometers operating between 23 and 89 GHz frequencies. These measurements started with

⁵⁴ See <http://www.jma.go.jp/jma/indexe.html>.

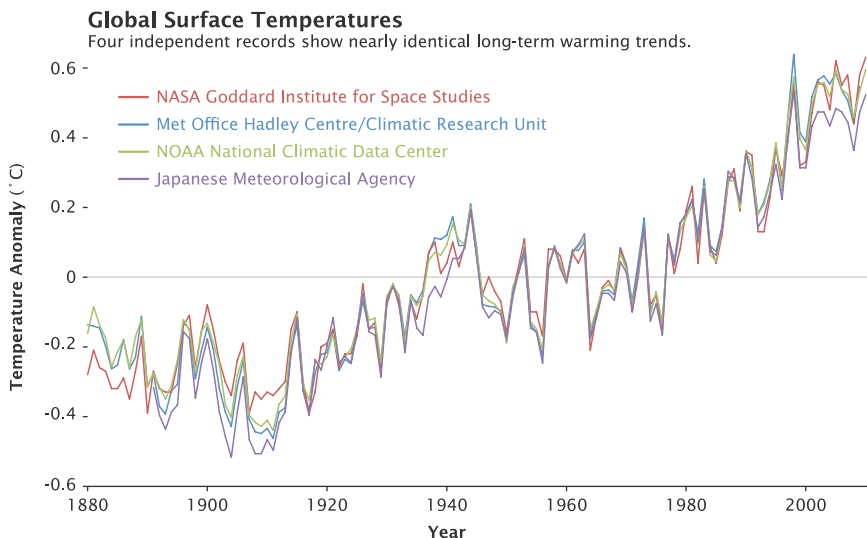


FIGURE A17-2 A comparison of the existing four global surface temperature datasets that are used in climate analyses. These datasets are based on the same input data and differ by interpolation among stations, treatment of missing data, and the length of the record. The data in this figure have been adjusted to a common baseline period.

SOURCE: Figure courtesy of Robert Simmon, NASA Earth Observatory, provided by Compton J. Tucker NASA Goddard Space Flight Center.

the microwave sounding-unit instruments and were followed by the advanced microwave sounding-unit instruments that began operation in 1988. As is not uncommon in science, early work using atmospheric temperature soundings produced a range of temperature trends. Recent work on atmospheric temperatures has shown no reasonable evidence of disagreement between these measurements and surface observations (Thorne et al., 2011).

Arctic Sea Ice

Satellite observation of sea ice is accomplished with a very high degree of accuracy. This results from spectral emissivity differences between open water (~ 0.4 – 0.5) and sea ice (~ 0.9 – 1.0) at a wavelength of 1.5 cm (Kwok, 2002). Furthermore, passive microwave radiometers are translucent to clouds, are weather insensitive, and operate from a polar orbit that provides near-daily observations of Arctic sea ice. According to the National Snow and Ice Data Center, all months (i.e., all Januaries, all Februaries, all Marches, etc.) from the late 1970s to 2010–2011 show declining Arctic sea ice with time.⁵⁵

⁵⁵ See <http://nsidc.org/arcticseaicenews/>.

Sea Level

Sea level is of direct interest to climate science because it varies directly with global mean temperature over short time scales. Temperature affects sea level through two mechanisms: (1) sea level rises through the thermal expansion of water as it warms, or it falls through thermal contraction of water as it cools; and (2) warmer global temperatures melt ice stored on land in glaciers and ice sheets, and the resulting ice loss raises sea level, while cooler global temperatures result in more water being stored on land in glaciers and ice sheets and sea level falls. Thus sea-level variations are an excellent, unambiguous indicator of planetary cooling or warming. For example, at the last glacial maximum, occurring before 20,000 years ago, sea level was >100 m lower than it is now (Clark et al., 2009). This huge quantity of water was stored on land in the form of glaciers and ice sheets (Lambeck et al., 2010).

Although tide gauges provide centennial-scale sea-level records from nearly 10 locations around the world, these few locations are insufficient for a global study of sea level. Researchers have also measured vertical accretion rates in salt marshes as a sea-level proxy, using radiocarbon, pollen, foraminifera, and other markers (reviewed in Mitchum et al., 2010). Since 1993, however, radar altimeters have measured sea level globally and directly with a high precision (Figure A17-3).

We have briefly reviewed global surface thermometer data, atmospheric temperature profile data from satellites, variations in Arctic sea ice, and sea-level data. All of these data unambiguously show the effects of increasing global temperatures.

Earth's Climate and the Sun

Since the late 1970s, the study of the Sun with instruments on satellites has progressed with continuous observations being collected. Lockwood and Frohlich (2007, 2008) have shown that the three mechanisms where the sun can influence the Earth's temperature (total solar irradiance, changes in the spectral distribution of solar irradiance, and the solar wind–magnetic field–cosmic ray–cloud hypothesis) have all been opposite to the observed increases in temperatures (Figure A17-4). The Sun's output is currently at record low values since the satellite era began in the late 1970s (Lockwood, 2009). Thus the Sun is not to blame for the observed global warming since the late 1970s to the present.

Projections of Warming Trends on Weather and Climate

Numerical simulation models of the Earth's weather and climate are called general circulation models because they simulate the circulation of the atmosphere. They are representations of the ocean, land, sea ice, and atmosphere where the Earth is a series of grid cells driven by energy, moisture, and pres-

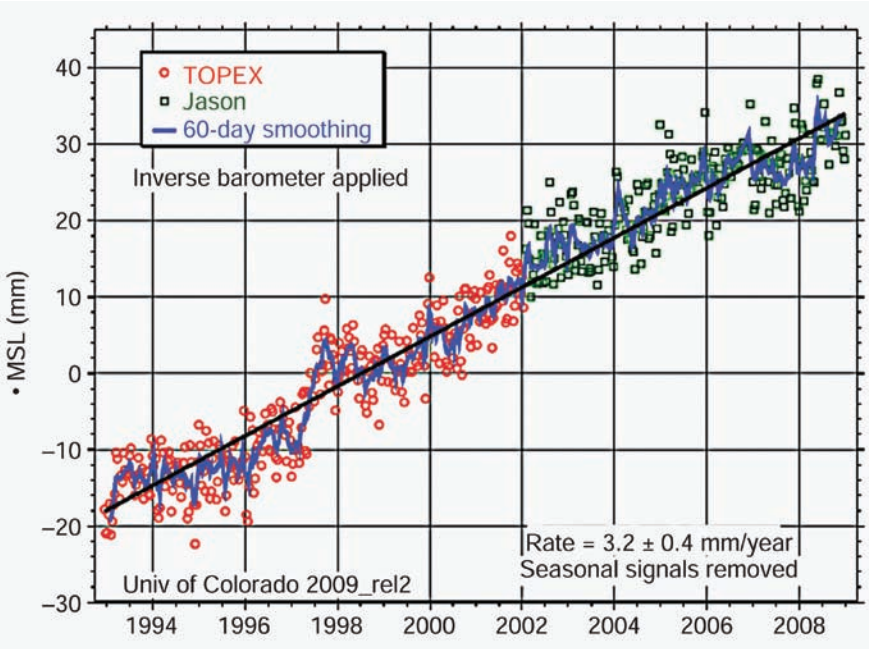


FIGURE A17-3 Sea-level rise based on radar altimeters from TOPEX and Jason, with seasonal variations removed.
SOURCE: Mitchum et al. (2010).

sure. Each grid cell interacts with adjacent cells horizontally and vertically to simulate climate (Figure A17-5). Model interactions are governed by systems of differential equations and incorporate climate-forcing factors such as land cover change, volcanic aerosols, and increasing greenhouse gas concentrations. Weather and climate models have been shown to be realistic at reproducing the global temperature and precipitation patterns of the 20th century and are widely used in weather and climate research (Delworth et al., 2006).

Climate model simulations, incorporating increasing greenhouse gas concentrations in the atmosphere, have been used to extrapolate precipitation patterns into the 21st century as surface temperatures increase. Several of these climate model simulation predictions can be described as “the wet getting wetter and the dry getting drier” (Held and Soden, 2006). The displacement of arid and semi-arid zones northward results from an expansion from the Hadley circulation cell under global warming (Figure A17-6) (Lu et al., 2007). These changes in climate have direct impacts on vegetation and biodiversity across the globe, including species range shifts, changing phenology, new invasive species, and new disease outbreaks (Parmesan and Yohe, 2003; Walther et al., 2002).

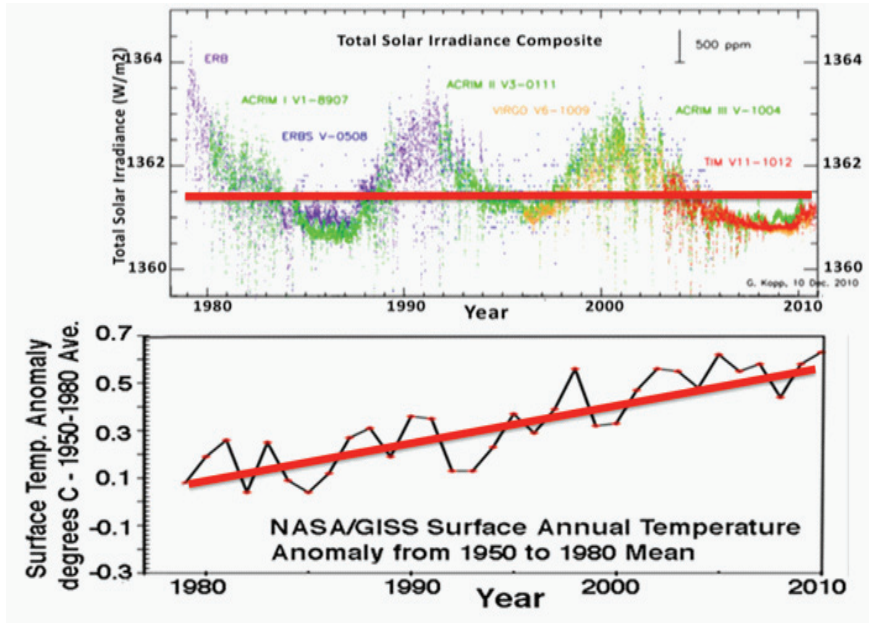


FIGURE A17-4 A comparison between the total solar irradiance (top) and the NASA/GISS surface temperature data (bottom), both from 1979 to 2010. This shows the sun is not responsible for the 1979 to 2010 increased surface temperatures.

Linkages Between Vector and Non-Vector Diseases and Climate

A variety of infectious diseases have been linked to variations in climate (reviewed in Patz et al., 2005). These include diarrheal diseases (Checkley et al., 1997), cholera (Colwell, 1996; Pascual et al., 2000), salmonella (Kovats et al., 2004), viral pneumonia (Ebi et al., 2001), hantavirus (Glass et al., 2002), influenza (Viboud et al., 2004), flea-associated plague (Parmenter et al., 1999), *Culicoides* biting midge-associated bluetongue (Baylis et al., 1999; Purse et al., 2005), African horse sickness (Baylis et al., 1999), mosquito-associated Murray Valley encephalitis (Nicholls, 1986), Ross River virus (Woodruff et al., 2002), dengue (Hopp and Foley, 2003; Linthicum et al., 2008), chikungunya (Chretien et al., 2007), malaria (Bouma and Dye, 1997; Bouma et al., 1996), and Rift Valley fever (Linthicum et al., 1999). Of these, we use the example of Rift Valley fever outbreaks to show the use of climate data to understand in what regions and at what times these disease outbreaks will occur.

The link between epizootics of Rift Valley fever and rainfall was first documented by Davies et al. (1985). Through an analysis of time-series rainfall data

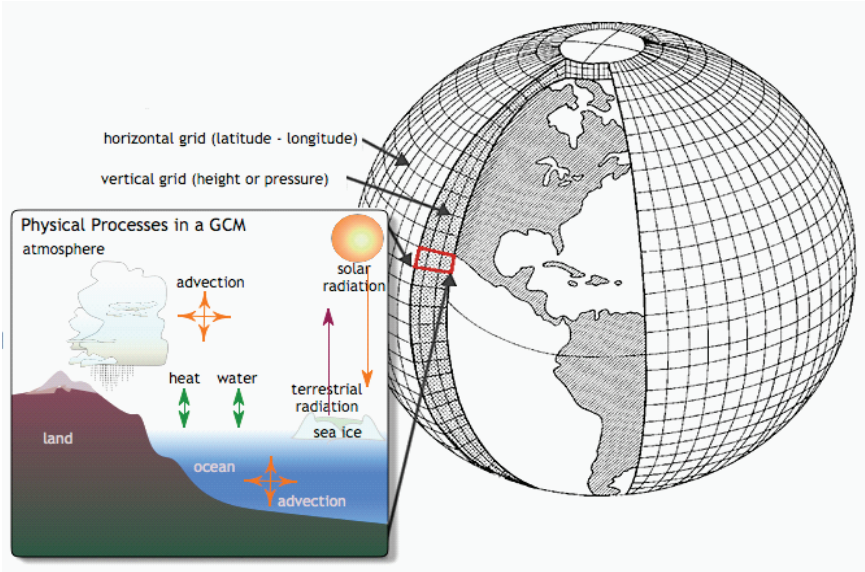


FIGURE A17-5 Representation of a general circulation model illustrating the grid cell nature of the model on the right, while on the left, many of the different important components of these models are shown.
SOURCE: Figure courtesy of the Center for Multiscale Modeling of Atmospheric Processes (CMMAP), Colorado State University, <http://www.cmmmap.org/learn/modeling/whatIs2.html>.

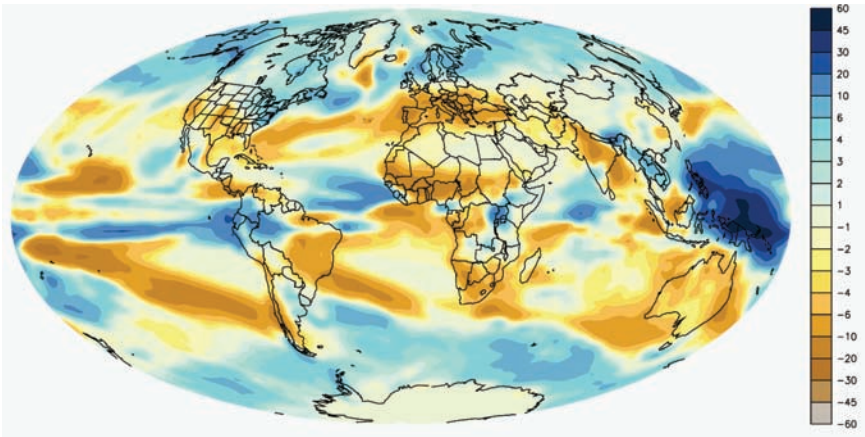


FIGURE A17-6 Change in precipitation between the 1971–2000 average and the 2091–2100 average in inches of liquid water/year (Held and Soden, 2006).
SOURCE: Geophysical Fluid Dynamics Laboratory, National Oceanic and Atmospheric Administration.

records from numerous stations in Kenya between 1950 and 1982, it was determined that periods with extended positive surplus rainfall corresponded to periods when Rift Valley fever epizootics occurred. Widespread, frequent, and persistent rainfall was shown to be a prominent feature of all epizootic periods. Heavy rainfall raises the level of the water table in certain areas, flooding grassland depressions that are the habitat of the immature stages of certain ground-pool-breeding mosquitoes of the genus *Aedes*. These findings have been collaborated by findings in southern Africa (Swanepoel, 1976) and West Africa (Bicout and Sabatier, 2004). Rift Valley fever virus is thought to be initially transmitted transovarially in these species. Under prolonged flooded conditions, large numbers of *Culex* species mosquitoes emerge and are an amplification vector for Rift Valley fever. Following the development of these conditions, Rift Valley fever first occurs in animals and subsequently in humans.

Linthicum et al. (1999) established that outbreaks of Rift Valley fever are closely coupled with above normal rainfall that is associated with the occurrence of the warm phase of El Niño/Southern Oscillation (ENSO) (Cane, 1983; Nicholson, 1986; Ropelewski and Halpert, 1987) and warm events in the equatorial western Indian Ocean (Anyamba et al., 2002; Birkett et al., 1999; Saji et al., 1999). Such warm ocean events precede by 2 to 3 months above normal and extended rainfall over East Africa, and are further enhanced when both the sea surface anomalies in the western Indian Ocean and equatorial central-eastern Pacific are synchronized. More than 90 percent of Rift Valley fever outbreak events since 1950 have occurred during warm ENSO events (Linthicum et al., 1999) (Figure A17-7). The interepizootic period is dominated by La Niña events (the cold phase of ENSO), which results in drought in East Africa and wetter than normal conditions in southern Africa (Anyamba et al., 2002; Nicholson and Entekhabi, 1986). Recent evidence shows that Rift Valley fever outbreaks in southern Africa are coupled with La Niña patterns (Anyamba et al., 2010). Interannual variability, in part driven by ENSO events with differential impacts on rainfall anomaly patterns in Eastern and Southern Africa, largely influences the temporal outbreak patterns of Rift Valley fever.

Our work on Rift Valley fever prediction thus uses climate data to inform us when and where regionally we should expect outbreaks. Subsequent detailed daily satellite observations identify where outbreaks will occur with a high degree of geographical specificity (~60 percent).

Prediction of Rift Valley Fever Outbreaks

Developed by Anyamba et al. (2002), prediction of Rift Valley fever outbreaks includes several components: (1) mapping of potential epizootic/epidemic regions through the combined use of satellite data, climate data, and historical reports; (2) closely following sea surface temperature anomalies with reference to phase and amplitude in the NINO 3.4 tropical Pacific and equatorial western

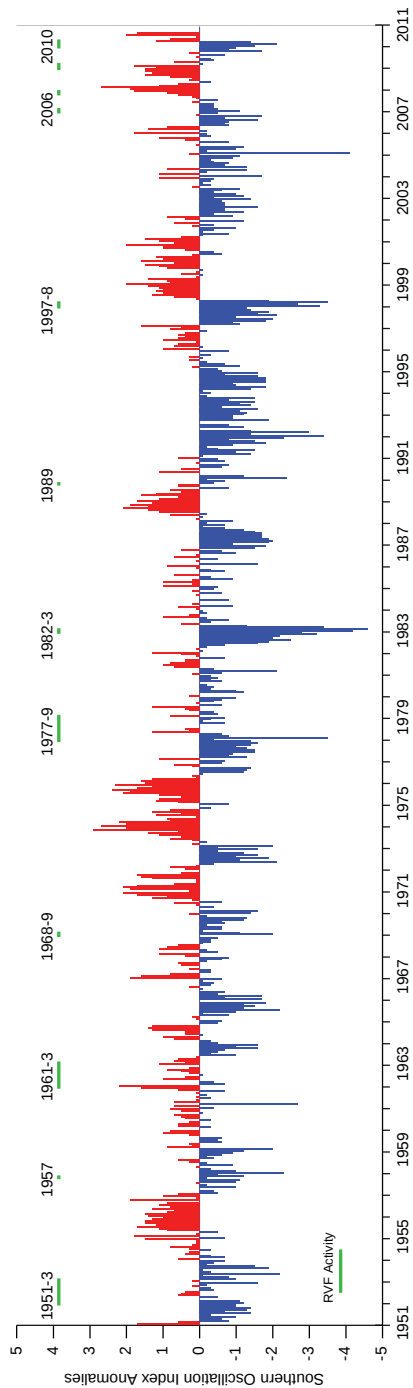


FIGURE A17-7 Rift Valley fever major outbreak events plotted against time and the Southern Oscillation Index, a measure of the phase of El Niño/Southern Oscillation events. Most Rift Valley fever outbreak events have occurred during the warm phase of ENSO (negative Southern Oscillation Index shown in blue).
SOURCE: Linthicum et al. (1999), and updated.

Indian Ocean areas; (3) monitoring patterns of outgoing long-wave radiation anomalies to infer and detect large-scale changes and shifts in the major atmospheric centers of tropical convection as a result of ENSO; and (4) monitoring patterns of normalized difference vegetation index anomalies over Africa as a proxy for excessive rainfall.

The first successful prediction using this system was made in 2006 (Anyamba et al., 2006, 2009) and provided a lead time of 3 to 4 months (Figure A17-8) to respond, although response and mitigation activities only started a month before the first reported outbreak. The predictions were subsequently confirmed by entomological and epidemiological field investigations of virus activity in the areas mapped to be at risk in Kenya, Somalia, and Tanzania with a geographic accuracy of 60 percent (Anyamba et al., 2009). Following the outbreak in East Africa, this system provided further predictions of outbreaks in Sudan in late 2007 and January 2008, 2009, and 2010 in southern Africa. These predictions and outbreak assessments are described in detail in Anyamba et al. (2010).

How Tools and Previous Approaches Could Have Relevance to Anticipating Conditions for Fungal Disease Emergence

We (Linthicum, Anyamba, and Tucker) have been studying the use of satellite data to predict Rift Valley fever outbreaks since the mid-1980s. Our study of Rift Valley fever occurrence led us to the antecedent role of high sea-surface temperatures in the tropical Pacific and western Indian Oceans that results in higher than average rainfall in East Africa, which triggers Rift Valley fever outbreaks. Alerted when the antecedent sea-surface temperature conditions are present, we then step up our near-real-time satellite data surveillance in East Africa that provides very specific location information for control measures to be put in place.

We propose a similar approach for anticipating conditions for fungal disease emergence: Use satellite data to map the abiotic conditions associated with fungal disease outbreaks; evaluate historical fungal outbreaks with respect to antecedent abiotic conditions; and use this knowledge to predict where and when future fungal outbreaks would occur.

A recent example how our prediction model could be applied elsewhere was the role that the very heavy 2011 summer rains have played in increased transmission of Murray Valley encephalitis virus, Ross River virus, and Kunjin virus in Australia (ProMed, 2011b). This same climate anomaly also produced widespread moist soil conditions and increased the likelihood of fungal diseases of cereal crops such as *Puccinia graminis* f. sp. *tritici* producing wheat stem rust (Figure A17-9), resulting in the release of warnings for the occurrence of fungal diseases in cereal crops in eastern and southern Australia (ProMed, 2011a).

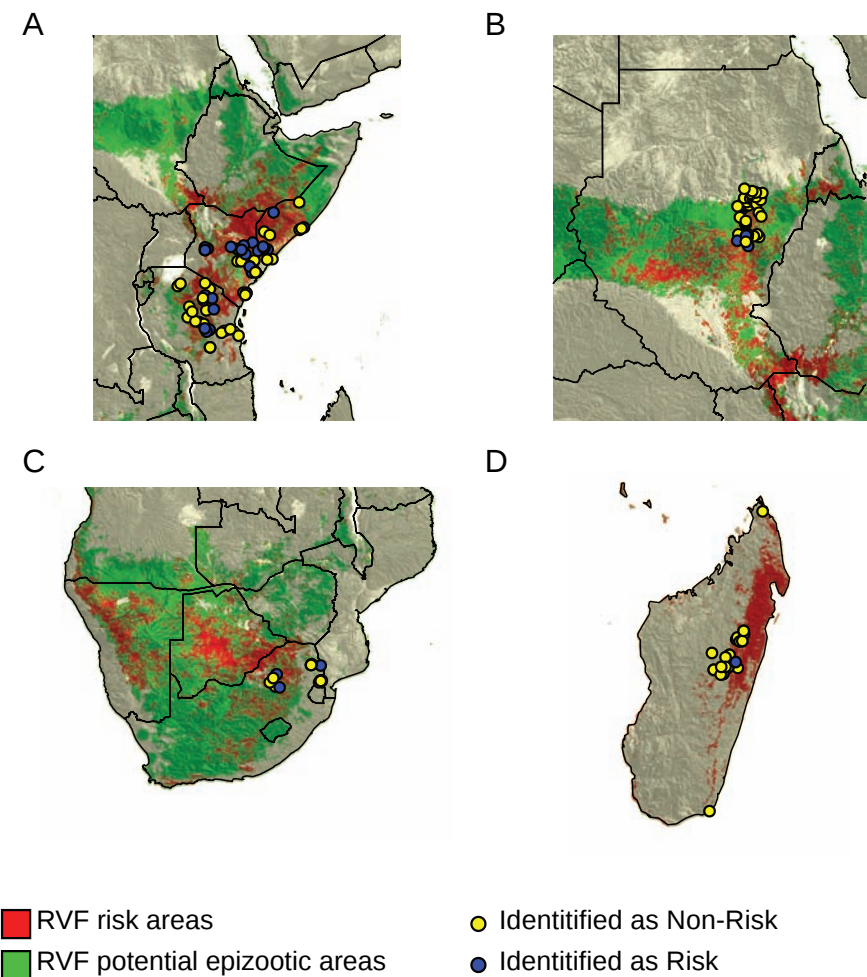


FIGURE A17-8 Summary Rift Valley fever (RVF) risk maps for (A) Eastern Africa: September 2006–May 2007; (B) Sudan: May 2007–December 2007; (C) Southern Africa: September 2007–May 2008; and (D) Madagascar: September 2007–May 2008. Areas shown in green represent Rift Valley fever potential epizootic areas. Areas shown in red represent pixels that were mapped by the prediction system to be at risk for RVF activity during the respective time periods. Blue dots indicate human cases identified to be with the Rift Valley fever risk areas, while yellow dots represent human cases in areas not mapped to be at risk.

SOURCE: Adapted from Anyamba et al. (2010).



FIGURE A17-9 Stem rust symptoms on wheat.

SOURCE: Agricultural research service, U.S. Department of Agriculture (http://www.ars.usda.gov/images/docs/9910_10104/stemrust_inset.jpg).

Tropical Glacier Recession, Amphibian Migration, and Subsequent Fungal Migration

Our group at NASA/Goddard Space Flight Center has documented New World tropical glacier variation from the mid-1980s to the present, including glaciers in the Cordillera Vilcanota in Peru. This heavily glaciated range, with multiple peaks over 6,000 m, is a key watershed for regional river systems, including the Amazon. Rapid environmental changes are documented in the region, including record tropospheric warming of 0.3°C per decade between 1974 and 1998 (Vuille et al., 2003), rise in freezing level (Diaz and Graham, 1996), and deglaciation (Bradley et al., 2006; Thompson et al., 2003). According to our current estimates, between the mid-1980s and mid-2000s, there has been approximately 30 percent glacial loss in this particular mountain range of southern Peru (Slayback and Tucker, in preparation). We have found warmer temperatures were largely responsible for tropical glacier recession in these areas (Figure A17-10). No variations in cloud cover or precipitation have been found, indicating global warming was the primary driver of glacial change.

Interdisciplinary research in the Cordillera Vilcanota, around Lake Sibinacocha, has been conducted for several years to investigate the impacts of climate change on local ecosystems (Seimon et al., 2009). This work includes research

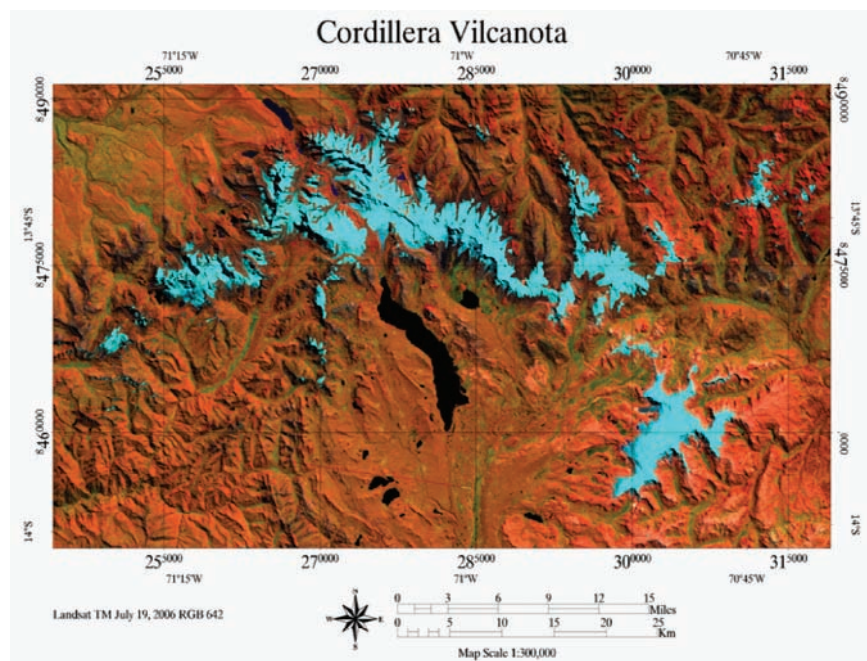


FIGURE A17-10 False-color Landsat satellite data (RGB 642) showing glaciers as the blue colors. The green colors represent green vegetation and the red colors represent areas of rock, sand, and soil.

SOURCE: Figure provided by Karina Yager, NASA-Goddard Space Flight Center.

on glaciers, vegetation, colonization of microbes in newly deglaciated soils, fossil plants, agropastoralism, species migration, and amphibian studies (Halloy et al., 2006; Nemergut et al., 2007; Seimon et al., 2007; Yager et al., 2010). Glacier retreat at higher elevations in the watershed has been rapid, resulting in the creation of new corridors and newly habitable areas for species migration and the upward range extension of numerous species, including plants, animals, amphibians, and pathogens.

Herpetologists on the team have documented the higher elevation colonization of three species of anurans—*Telmatobius marmoratus*, *Rhinella spinulosa*, and *Pleurodema marmorata*—that have expanded their ranges and moved to unprecedented elevations for amphibians (5,200–5,400 m) into new lakes and ponds created by recent deglaciation (Seimon et al., 2007). In the case of *P. marmorata*, climatic warming has resulted in an approximate 200 m vertical increase in its range, corresponding to the amount of glacier retreat since 1880 (Seimon et al., 2007).

These amphibian species are opportunistic in their adaptation to the warming climate by migrating to and spawning in ever-higher terrain. However, new climate conditions are also proving advantageous for the spread of epidemic disease, and in particular Chytridiomycosis. This pathogenic chytrid (*Bd*) produces aquatic zoospores on amphibian skin, and under certain conditions becomes a highly lethal infection (Seimon et al., 2005). Chytrid fungus has been linked to amphibian population declines and even species extinction across the globe (Daszak et al., 1999; Pounds et al., 2006).

New challenges are being presented for the long-term survival of amphibian species in this watershed with climate change. Since 2003, a year after *Bd* was first detected in this region, all three species have been decreasing in number, and *T. marmoratus* has not been documented in the Sibinacocha watershed since 2005 (Seimon et al., 2007; Yager et al., 2010). The current research indicates that recent warming, and intense solar heating of glacier ponds during the day, may be contributing to the ability of chytrid to expand and thrive at unprecedented altitudes and terrain (Seimon et al., 2007). In addition, as the glaciers continue to melt, ponds that were once inhabited by amphibians are experiencing a reduction in meltwater or are drying up altogether, leading to loss of habitat and contributing to subsequent population declines. Amphibians are some of the most sensitive species to environmental changes, and are becoming more susceptible to life-threatening disease and possible extinction under current climate patterns.

Conclusions

The Earth's climate is warming and our Sun is not responsible. Weather and climate simulation models project even warmer temperatures by the middle of this century, with some areas getting wetter and others drier. These changing patterns of temperature and precipitation will alter endemic areas for various plant and animal diseases, including fungal pathogens. We reviewed how knowledge of climatic linkages is being used to predict the outbreak regions of Rift Valley fever in Africa, complemented by detailed satellite observations to identify specific locales where control measures should be undertaken. We advocate a similar approach to identify areas where fungal diseases may emerge: understand the biology of specific fungal pathogens; use satellite data to establish temperature and precipitation climatology in the areas of interest; associate this information with documented fungal outbreaks; and use this knowledge in conjunction with satellite data to predict the impacts of a changing and variable climate on fungal pathogens.

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A18

HOST-PATHOGEN DYNAMICS OF AMPHIBIAN CHYTRIDIOMYCOSIS: THE ROLE OF THE SKIN MICROBIOME IN HEALTH AND DISEASE

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Abstract

Amphibian biodiversity is currently facing a severe crisis having recently experienced declines in 42 percent of all species, and as many as 32 percent are

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threatened with imminent extinction. The most alarming extinctions and declines have occurred enigmatically in protected, apparently pristine habitats. An emerging infectious disease, chytridiomycosis, is directly linked to the recent extinction or serious decline of hundreds of amphibian species and is increasingly proposed as a primary threat to amphibians. Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), infects the skin of amphibians and has been described as causing the greatest loss of vertebrate biodiversity due to disease in recorded history. The severity of the current amphibian biodiversity crisis suggests that *Bd* is a fundamentally new challenge to amphibians from previous global and environmental changes. While many amphibian species are susceptible to this fungal pathogen, others are silent carriers exhibiting no signs of disease. How amphibian hosts survive with *Bd* infection is still unknown; however, host immunity, differences in pathogen virulence, and environmental differences that may limit growth and reproduction of the pathogen have all been proposed as possible mechanisms that could lead to *Bd*-infected host survival. Here we examine amphibian declines in one of the best-documented systems, the Sierra Nevada of California, and review the role that the amphibian skin microbiome may play in host–pathogen dynamics of the chytridiomycosis–amphibian host system.

Introduction

The amphibians are long-term survivors (existing on earth for more than 350 million years) that endured four previous mass extinctions (e.g., 95 percent of all living species were lost in the Permian-Triassic extinction) (Wake and Vredenburg, 2008). Through these extinctions, not only did all three orders of amphibians escape extinction, but most families and genera survived (Wake and Vredenburg, 2008). Today, the amphibians, presently including more than 6,800 species (AmphibiaWeb, 2011), are the most threatened group of vertebrates with over 40 percent of species in decline and over 30 percent threatened with extinction (Stuart et al., 2004). There are many potential causes for the declines, but an emerging infectious disease, chytridiomycosis (Longcore et al., 1999), caused by the infectious fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) is the most alarming (Daszak et al., 2000). This fungal pathogen is associated with the recent decline or extinction of more than 200 species of amphibians (Fisher et al., 2009; Skerratt et al., 2007). Epizootic waves of chytridiomycosis have been identified in Australia, Central and South America, and the western United States (Fisher et al., 2009). In each case the epizootic caused mass mortality and collapse of amphibian faunas (Berger et al., 1998, Lips et al., 2006, Vredenburg et al., 2010a). Once *Bd* invades naïve amphibian host populations, they can collapse very quickly. In Panamanian sites, 50 percent of species were extirpated 4 to 6 months after *Bd* invaded (Lips et al., 2006). The surviving species declined in population size by 80 percent (Lips et al., 2006). In California, a moving epizootic wave of *Bd* is causing the collapse of entire metapopulations of ranid frogs

in some of the most protected and pristine areas in the United States (Vredenburg et al., 2010a). In Australia, most of the damage was done before the pathogen was identified (Laurance et al., 1996), but post hoc studies estimate that *Bd* was responsible for the decline and disappearance of a majority of amphibian species along the diverse eastern tropical montane areas (Fisher et al., 2009).

The Decline of the Mountain Yellow-legged Frog in the Sierra Nevada of California

The dramatic decline of California's mountain yellow-legged frog (a species complex consisting of *Rana muscosa* and *Rana sierrae*; Vredenburg et al., 2007) is emblematic of global amphibian declines (Stuart et al., 2004). Historically, lakes in California's Sierra Nevada were often inhabited by hundreds of frogs and thousands of tadpoles (Grinnell and Storer, 1924) of this diurnal and highly aquatic taxon (Vredenburg et al., 2005). By 1997, despite the fact that the majority of their habitat is fully protected, *R. sierrae* and *R. muscosa* had disappeared from 93 percent and 96 percent, respectively, of their historic range (Vredenburg et al., 2007; Figure A18-1). As a consequence of this decline the two species of mountain yellow-legged frog have gone from being the most common vertebrates in the Sierra Nevada (Grinnell and Storer, 1924) to species classified as "critically endangered" (Stuart et al., 2004). These two species are not the only ones in trouble in the protected landscape of the Sierra Nevada. In fact, five of the seven species of amphibians that occur in the high-elevation areas of the Sierra Nevada (>2,000 m above sea level) are threatened in the range (*R. muscosa*, *R. sierrae*, *Bufo canorus*, *B. boreas*, and *Ambystoma macrodactylum*), while only two species (*Hydromantes platycephalus* and *Pseudacris regilla*) are thought to have stable populations (Stebbins and Cohen, 1995).

The declines of amphibians that occur in high-elevation protected habitats around the world have been described as enigmatic (Stuart et al., 2004). Decline hypotheses in these areas include the negative effects of increasing UV radiation (Blaustein et al., 1994), pesticide drift (Davidson, 2004), introduced species (Vredenburg, 2004), climate change (Pounds et al., 1999), and disease (Longcore et al., 1999). In the Sierra Nevada, most studies have focused on the decline of the two species of yellow-legged frog. To date, studies have not directly addressed climate change and have shown no evidence for UV radiation effects (Vredenburg et al., 2010b) or pesticide drift (Bradford et al., 2011), but they have shown large negative effects from introduced species (Knapp and Matthews, 2000) and disease (Vredenburg et al., 2010a). Like many montane areas worldwide, the vast majority of the high-elevation portion of the Sierra Nevada, consisting of more than 15,000 glacial lakes, was historically fishless (Knapp and Matthews, 2000). Despite the fact that most of the habitat is protected from habitat destruction, humans changed the landscape dramatically by planting non-native fishes. By 1997 most of the lakes and streams in the area contained self-reproducing

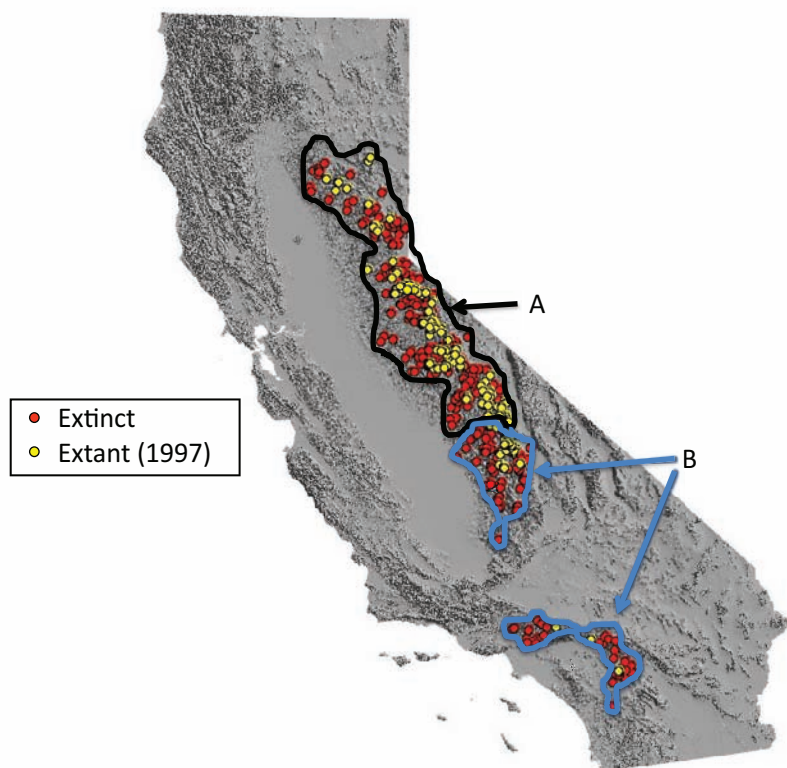


FIGURE A18-1 Decline of (A) Sierra Nevada mountain yellow-legged frog, *Rana sierrae*, and (B) southern mountain yellow-legged frog, *Rana muscosa*, in California, USA. Yellow points indicate extant populations in 1997 and red points indicate extinct populations compared to historically documented sites.
SOURCE: Adapted from Vredenburg et al. (2007).

non-native trout, which have contributed significantly to the decline of the two species of yellow-legged frog (Knapp and Matthews, 2000; Vredenburg, 2004). In 2004 a whole-lake experimental study determined that predation on tadpoles by introduced trout was causing frog population extirpations (Vredenburg, 2004). The same study also demonstrated that threatened frog populations would recover quickly when habitat was restored by removing the non-native fish (Vredenburg, 2004). Additional research showed that habitat restoration (to the fishless condition) worked across the mountain range for both yellow-legged frog species (Knapp et al., 2007). Unfortunately, further research ultimately revealed that amphibian declines in the Sierra Nevada, perhaps like other areas around the world, are complex in nature and can be caused by multiple factors that may act

synergistically. Although it was not understood until 2010, an infectious fungal disease (chytridiomycosis) was also causing population collapse of the yellow-legged frog (Vredenburg et al., 2010a), but it just happened that fish-removal studies occurred ahead of the epizootic wave.

Differential Outcomes for Frog Host Populations After Pathogen Invasion

Chytridiomycosis is caused by the waterborne fungal pathogen *Batrachochytrium dendrobatidis*, whose only known hosts are larval and adult amphibians. This pathogen was first described in the late 1990s (Berger et al., 1998; Longcore et al., 1999) and is now known to inhabit six continents (Skerratt et al., 2007). The infective stage is a free-living flagellated zoospore that encysts in the skin of an amphibian and develops into a zoosporangium. Zoosporangia produce zoospores via asexual reproduction (sexual reproduction may also occur; Morgan et al., 2007) that are released into the water through a discharge tube (Berger et al., 2005). Tadpoles of most species are minimally affected by chytridiomycosis and the effects on frogs are highly variable, with some species succumbing to the disease within weeks and others experiencing few negative effects (Fisher et al., 2009; Skerratt et al., 2007). Chytridiomycosis likely causes frog mortality by severely disrupting epidermal functions and leading to osmotic imbalance (Voyles et al., 2009).

Despite a growing literature on chytridiomycosis (Fisher et al., 2009; Rosenblum et al., 2010), we have little predictive power in assessing why *Bd* presence in some areas causes epizootic die-offs while in other areas amphibian hosts survive *Bd* infections in an apparently enzootic state for years with little or no effect on host population survival (Briggs et al., 2010). Our studies of yellow-legged frogs in California offer one of the most complete insights into the host–pathogen dynamics of chytridiomycosis (Briggs et al., 2010; Vredenburg et al., 2010a). Along with an important study from Central America (Lips et al., 2006), we show that *Bd* epizootics occur when *Bd* invades completely naive host populations. We describe the dynamics of *Bd* and its spread into three naive metapopulations of yellow-legged frogs, each consisting of collections of connected subpopulations ($n = 80$) within widely separated drainage basins in Sequoia and Kings Canyon National Park (SEKI). Our study was the first to capture the spread of *Bd* on a small scale (more than hundreds of kilometers) and provided insights into how the pathogen spreads between host populations. The *Bd* epizootic moved linearly across the landscape at approximately 700 m per year. In one basin (Sixty Lake Basin, Figure A18-2, panels F–J), *Bd* took 4 years to invade all of the subpopulations (Figure A18-2) and we proposed that *Bd* was probably invading new host populations through movement of frog hosts as opposed to movement by water (movement was upstream) or by birds or other flying organisms that might carry *Bd*. (*Bd* always infected the nearest uninfected host population.) With the data we collected from 1996 to 2008, we demonstrate that *Bd* invaded naive host

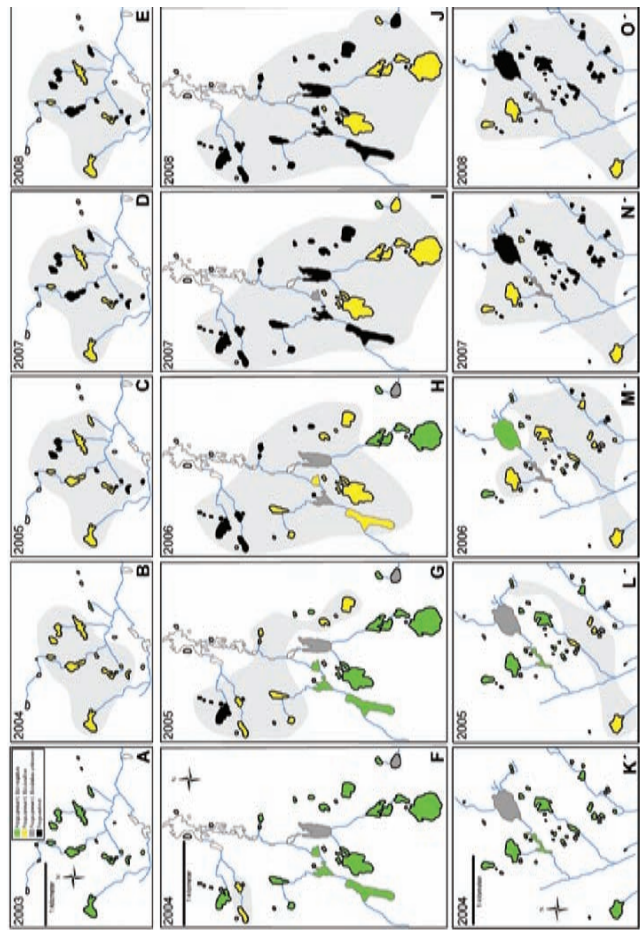


FIGURE A18-2 Maps of the three study metapopulations showing the spread of *Bd* and frog population status (adults only) during a 4-year period following the initial detection of *Bd*. Depicted are (A–E) Milestone Basin, (F–J) Sixty Lake Basin, and (K–O) Barrett Lakes Basin. Lake color (green, yellow, and black) shows the *Bd* infection and frog population status, and the light gray shaded region surrounds the area in which frog populations were *Bd*-positive in each year. Lakes shown with a thick black outline are fishless, and a thin gray outline indicates that non-native fish were present. The infection status of frog populations depicted in panels A and K is based on mouthpart surveys of 459 tadpoles. The infection status of frog populations in panels B–J and L–O is based on 4,591 skin swabs analyzed using a real-time PCR assay. SOURCE: Vredenburg et al. (2010a).

populations of frogs and that infection prevalence quickly reached 100 percent (Figure A18-3, panel B), but mass mortality and host population collapse did not occur until the average *Bd* infection load (or intensity of infection on individuals) for a population reached more than 10^4 zoospore equivalents (Figure A18-3, panels A and C; Vredenburg et al., 2010a). This condition was later named the “Vredenburg 10,000 Zoospore Rule” (Kinney et al., 2011) and was shown to also predict mortality in neotropical salamanders (Cheng et al., 2011).

In the Sierra Nevada, infection by *Bd* did not always lead to epizootic events and host population collapse. About 100 km north of our SEKI study area in and around Yosemite National Park, we describe the *Bd*–frog dynamics under stable enzootic conditions (Briggs et al., 2010), which contrast sharply with conditions we describe above in the same species of yellow-legged frog in SEKI (Vredenburg et al., 2010a). We discovered that in the Yosemite area, frog host population dynamics are strikingly different and that host populations survive, at least for a decade, despite infection by *Bd* (Briggs et al., 2010). We determined that while infection prevalence (proportion of infected hosts in a population) could be high (more than 60 percent), the infection intensity of hosts remained low (Briggs et al., 2010), well below the Vredenburg 10,000 Zoospore Rule (Kinney et al., 2011). In fact, several other studies have found that host amphibians do not die when *Bd* infection intensities remain low (Cheng et al., 2011; Kinney et al., 2011; Kriger et al., 2007; Retallick et al., 2004). Why the dynamics of the host–pathogen interaction is so different in different sites and among different species remains a mystery, but we believe the key to host survival is the low infection intensities on individual host frogs (Briggs et al., 2010; Kinney et al., 2011; Vredenburg et al., 2010a) and salamanders (Cheng et al., 2011).

Mutualistic Bacteria Play a Role in Amphibian Resistance to Fungal Disease

There are several factors that may prevent fungal infection intensities from reaching a lethal threshold in amphibian hosts, such as exposure to a thermal environment that limits *Bd* growth (Piotrowski et al., 2001), immune response by the host (Ramsey et al., 2010; Rollins-Smith et al., 2000), or density-dependent host–pathogen dynamics (Briggs et al., 2010). Another possibility is that a host’s microbiome may disrupt fungal invasion and growth; in particular, species of mutualistic bacteria may be able to produce compounds that deter fungal colonization and growth. The idea that microbial symbionts may protect amphibian hosts from fungal pathogens was discovered, in a sense, by accident. Until relatively recently, it was assumed that amphibians had few microbes living on their skin since it is generally accepted that amphibians wear their defenses, like armor, on the outsides of their bodies (Duellman and Trueb, 1986). Amphibians are distinct among vertebrates in that they contain specialized skin secretion glands (granular glands) that produce toxins, presumably to deter predators (Duellman

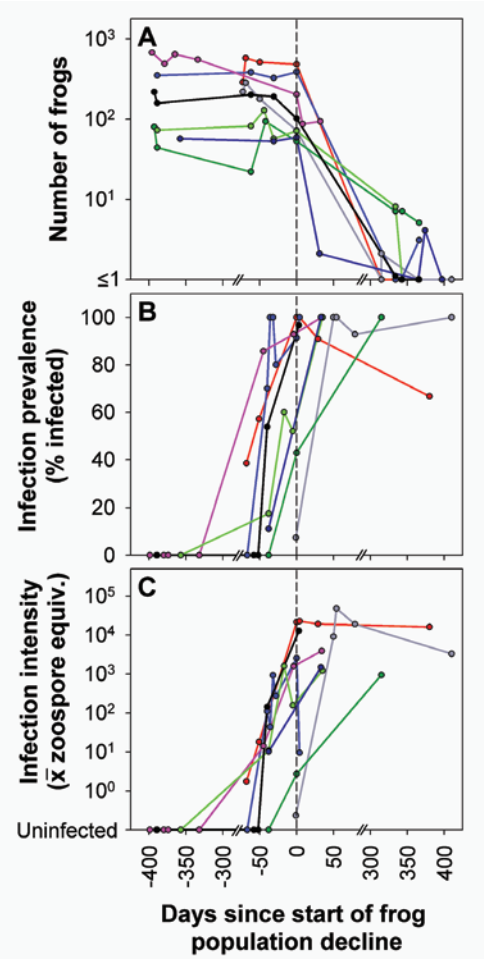


FIGURE A18-3 Frog-*Bd* dynamics in eight intensively sampled populations in Milestone and Sixty Lake basins before and after detection of *Bd*: (A) frog counts (adults + subadults) from visual encounter surveys; (B) infection prevalence, defined as the fraction of skin swabs collected from each population on each date positive for *Bd*; and (C) infection intensity, defined as the average zoospore equivalents on swabs collected from each population on each date. Data are from frog populations that were sampled more than once per year, that experienced more than 80 percent declines by the end of 2006, and for which the decline in the number of frogs was greater than 10. This last criterion excluded populations that were very small before *Bd* arrival. Populations are aligned along the x axis such that “0” represents the date on which each frog population began to decline. This was calculated for each population by determining the date at which the number of postmetamorphic frogs dropped below 20 percent of the average population count before that point. SOURCE: Vredenburg et al. (2010a).

and Trueb, 1986). Many species are well known for this characteristic, such as the poison dart frogs of the neotropics (genus *Dendrobates*) that produce neurotoxins strong enough to incapacitate monkeys hit by blow-darts from hunters who rub the darts on the skin of the frogs, or the toxic newts (genus *Taricha*) that produce the same chemical defense compounds, known as tetrodotoxins, found in deadly puffer fish.

Amphibians, although generally tied to water, have colonized many diverse habitats, including many with no standing or running water (Duellman, 1999). In fact, many diverse species have developed reproductive modes that free them in some capacity or completely from water (e.g., direct developers). The four-toed salamander (*Hemidactylium scutatum*), although not a direct developer, is of interest for this chapter because the female will lay her eggs outside water along steep banks or edges of ponds and she will brood them or guard them until they hatch as larvae. Originally it was presumed that the female was guarding her eggs against egg predators, but behavioral experiments showed that unattended eggs were not eaten by egg predators; in fact, the eggs were unpalatable to many predators (Hess and Harris, 2000), and died instead from fungal infections. Egg-brooding females protect their eggs by inoculating them with colonies of beneficial bacteria (Harris et al., 2006). A laboratory study later showed that the bacterium, *Janthinobacterium lividum*, cultured from female four-toed salamanders inhibited the growth of *Bd* (Harris et al., 2006).

Could the information from egg-brooding salamanders in the eastern United States provide insight into why some populations of yellow-legged frogs in California survived *Bd* infection while others did not? In 2005 we collected bacterial cultures from susceptible and nonsusceptible populations of *Rana muscosa* and *R. sierrae*. We found that individual frogs from nonsusceptible populations were more likely to contain culturable populations of the symbiotic bacterium *Janthinobacterium lividum*, the same species of bacteria discovered on the four-toed salamander (Woodhams et al., 2007). A laboratory study to test whether inoculations of *J. lividum* could protect susceptible *R. muscosa* from *Bd* infections was then initiated. Adult *R. muscosa* were collected from the wild in SEKI in an area ahead of the *Bd* epizootic wave and were transported to the Harris Laboratory at James Madison University. The laboratory experiment consisted of three treatments of frogs: one group received *J. lividum* only, another group received *Bd* only, and a third was first inoculated with *J. lividum* and was then infected with *Bd*. As expected, all of the frogs in the *Bd*-only treatment died from chytridiomycosis within several weeks, but the frogs inoculated with *J. lividum* before being infected survived, as did the frogs that only received inoculations of *J. lividum*, providing evidence that bacterial symbionts could alter the outcome of *Bd* infections in susceptible host populations (Harris et al., 2009).

Could Bioaugmentation of the Skin Microbiome on Host Frogs Make *Bd* Epizootics Less Deadly?

The successful laboratory experiment with yellow-legged frogs showed that inoculations with *Janthinobacterium lividum* can effectively inhibit *Bd* growth and colonization (Harris et al., 2009). The production of violacein, an antifungal compound (Brucker et al., 2008), appears to be the mechanism used by *J. lividum* to combat the colonization of *Bd* (Harris et al., 2009). Our field cultures from admittedly limited *Rana muscosa* and *R. sierrae* populations indicated that a small proportion of individual frog hosts even in susceptible populations did naturally include *J. lividum* in their skin microbiome (<10 percent compared to >80 percent from susceptible and nonsusceptible populations of hosts, respectively; Lam et al., 2010). Nevertheless, because the *Bd* epizootic was extirpating hundreds of yellow-legged frog populations, we decided to initiate a field bioaugmentation experiment of *J. lividum* on frogs at a *Bd* epizootic site in the Sierra Nevada. We planned a replicated whole-lake experiment in the spring of 2010 at a site called Dusy Basin in the northeasternmost section of Kings Canyon National Park. This site represented some of the very last susceptible populations of *R. sierrae* that had yet experienced the *Bd* epizootic by 2009. By the time we arrived after the spring snowmelt in 2010, there was only one population in Dusy Basin of *R. sierrae* left containing living adults; all other frogs in the basin were dead, presumably killed by *Bd*.

In July 2010 we captured 40 adult *R. muscosa* at a single lake in Dusy Basin and collected bacteria culture samples. Of the 40 frogs sampled, only one successfully resulted in a bacterial culture of *J. lividum*. We used this single culture to grow large volumes of *J. lividum* and returned 2 weeks later to Dusy Basin to inoculate as many frogs as we could find with live cultures of *J. lividum*. We captured 100 adult frogs at the single remaining population in the basin and treated 80 with bacterial baths, leaving 20 as controls. All frogs were marked individually with microchips (PIT tags) and were treated equally in terms of handling time. Control frogs were soaked in pond water from the lake while treatment frogs were soaked in provosoli solution containing live populations of *J. lividum* in broth. Each animal was weighed, measured, swabbed (for *Bd* PCR assay; Boyle et al., 2004; Hyatt et al., 2007), and released. We then revisited the site five times in the remaining days of summer, capturing as many frogs as we could find. Each frog was checked for a microchip, weighed, swabbed, and released. By the end of summer, 6 control frogs had been recaptured and 62 bacterially treated frogs had been recaptured. The highest *Bd* infection intensities were recovered on the control frogs, several control frogs contained infections with >10,000 zoospore equivalents, a lethal infection for the species (Vredenburg et al., 2010a). Due to the high elevation of the site (~3,352 m) and the extremely large amount of precipitation during the winter of 2010–2011, we are not able to return to the site until mid-July 2011. At this time we have no way of knowing what the fate of the population may be. If the microbiome bioaugmentation technique is successful,

it holds great hope for the conservation of susceptible populations of amphibians globally. If the technique does not work, then we must continue to try to understand the factors that may keep *Bd* infection intensities on individual host amphibians low, because we currently have no way of controlling the spread of this highly virulent pathogen.

Acknowledgments

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A19

THE EFFECT OF TRADE-MEDIATED SPREAD OF AMPHIBIAN CHYTRID ON AMPHIBIAN CONSERVATION

Ché **Weldon**⁵⁹ and Matthew C. **Fisher**⁶⁰

Introduction

Accelerating losses of amphibian biodiversity were brought to the forefront of conservation concerns in 1989 at the First World Congress of Herpetology, and the National Research Council Workshop the following year (Blaustein and Wake, 1995). Although obvious causes of amphibian declines were known (e.g., habitat destruction and alteration, introduction of exotic species, pollution), the causes of some declines and disappearances were undetermined, such as enigmatic declines in tropical Australia, Meso-America, and western United States. Detailed retrospective studies of declines in California national parks (Drost and Fellars, 1996) and Monteverde Cloud Forest Preserve in Costa Rica (Pounds et al., 1997) gave conclusive evidence of community-wide declines. Amphibian species have disappeared or declined at such an alarming rate over the past few decades that the phenomenon is now considered to be an amphibian extinction crisis (Mendelson et al., 2006; Wake and Vredenburg, 2008).

In 2004, the Global Amphibian Assessment (GAA) conducted by the International Union for Conservation of Nature (IUCN) revealed that 32.5 percent of the world's ca. 6,000 amphibian species are currently threatened with extinction, and more than 120 have already disappeared (Stuart et al., 2004). Furthermore, half of 435 rapidly declining species were threatened by enigmatic processes (e.g., disease, climate change) and disease was detected in a significant proportion of families (15/36 families) with threatened species (Stuart et al., 2004). While long suspected, little direct evidence for the emergence of infection existed until a new pathogen to science, the fungal chytrid *Batrachochytrium dendrobatidis* (*Bd*), was found in dead and dying frogs collected in the mid-1990s from declining populations in Australia and Panama (Berger et al., 1998; Longcore et al., 1999).

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Many amphibian declines considered at the time to be the cause of introduced species or anthropogenic activities are now known to have been partly due to chytridiomycosis (Green et al., 2002; Muths et al., 2003). More recently, *Bd* has been identified as a rapidly emerging amphibian pathogen, capable of acting as both the proximate and ultimate cause of amphibian extinction (Fisher et al., 2009a; Schloegel et al., 2006).

In this article, the contribution of the amphibian–chytridiomycosis spread pathway to amphibian declines is investigated, and conventional as well as novel approaches to amphibian conservation with regard to disease threat are evaluated. The essay argues for greater collaboration among science, conservation, and policy if the chytridiomycosis panzootic is to be warded off more efficiently, and summarizes possible strategies to meet that goal.

The Chytridiomycosis–Amphibian Spread Pathway

Through trade and travel, humans introduce species both intentionally and inadvertently. There is a lucrative global trade in amphibians for purposes ranging from experimental animals to cuisine delicacies to pets. The GAA reported that there are 278 amphibian species in pet trade, with the main centers for export being the wet tropics (Stuart et al., 2008). The commercial collection of wild amphibians has often been unsustainable, resulting in reductions in even highly protected amphibian populations such as the Japanese Giant salamander and the Mountain Chicken frog of the islands of Montserrat and Dominica (Anita et al., 2007; Wang et al., 2004).

The conservation threat of traded species affects local and endemic species when introduced exotic and non-native species become invasive and establish feral populations. In addition, diseases and parasites are often carried along unintentionally with their hosts, allowing them to cross geographic boundaries that historically contained these agents at their source of origin (Cunningham et al., 2003). Not many pathogens become established with invasive species, but those that do have the potential to seriously threaten native wildlife (Lyles and Dobson, 1993). Amphibians are known for their ability to become invasive, as demonstrated by the Invasive Species Specialist Group (ISSG) list of 100 of the world's most invasive alien species, which includes three amphibians (ISSG, 2008). Also listed is *Bd*, for which invasive amphibians are now known to be the primary vectors of spread (Garner et al., 2006). In Australia, for example, *Bd* is found among others in Cane toads, a recently introduced invasive alien species (Fisher and Garner, 2007). This stands as a testimony to the complexity of biological invasion issues where not only is *Bd* an invasive alien species, but its spread is facilitated by an invasive alien toad. Thus, aspects of the disease impacts on amphibian populations around the world result from the unintentional introduction of a pathogen as an invasive alien species. This leads to a paradox in conservation management, namely that in order to contain the pathogen threatening the organ-

isms targeted for conservation (e.g., amphibians), the very group conservationists are trying to protect need to be mitigated to prevent the pathogen from spreading.

The disease pathway is not always a single invasive host–pathogen system, but may involve multiple host species that are either directly or indirectly related to the source population. Several invasive frog species play a role in the exportation of *Bd* from South Africa and its ongoing spread and impact on amphibian populations around the world. Trade in African clawed frogs (*Xenopus laevis*) since the 1930s was identified as one of the first human-mediated pathways for spreading *Bd* internationally (Weldon et al., 2004). Feral populations that became established in countries where the species had been moved (Weldon et al., 2007) allowed for potential transmission of *Bd* to naïve amphibian species. Such a link was hypothesized to exist between *X. laevis* and the American bullfrog *Rana catesbeiana*, an invasive species and major vector of the disease (Daszak et al., 2004; Weldon et al., 2004). Various other strong links have been confirmed between the international amphibian trade for use in the food, pet, and laboratory industry, and the global dispersal of *Bd* (Fisher and Garner, 2007; Garner et al., 2006; Schloegel et al., 2009). A second species traded from South Africa, *Xenopus gilli*, was identified as the source of a *Bd* infection transmitted to captive *Alytes muletensis* in Spain (Walker et al., 2008). These infected toads were later reintroduced into their native habitat on the island of Mallorca, thus introducing the pathogen into this disease-naïve ecosystem. What makes this case even more extraordinary is that *Bd* was transmitted from one critically endangered species to another, dismissing the assumption that the more common, widely distributed species are mostly responsible for disease spread. This case study demonstrates the caution with which reintroduction programs should be approached when there is a potential for disease organisms to be vectored alongside their hosts; it is now clear that invasive frogs and toads have spontaneously exposed many native species of amphibians around the world to *Bd*, often with catastrophic results.

Conventional Amphibian Conservation Strategy

Conventional approaches to amphibian conservation focus on mitigating factors that have been threatening amphibian populations for more than a century. Primary threats that have traditionally received conservation attention include habitat loss and fragmentation, overexploitation, and species invasion (Stuart et al., 2008). Because disease is a relative newcomer on the scene of threats to amphibian biodiversity, it has traditionally received little conservation attention and proven mitigation measures are generally underdeveloped.

A common approach to conserving species is to make habitat or site the conservation priority as some form of protected area. The species that require protection are then viewed as important features that are present at that site and that qualify it for a particular protected-area designation status (Tucker, 2005). However, no amphibian species is protected from becoming infected with *Bd* by

the site conservation designations approach because the distribution of disease agents is not restricted by political boundaries. This observation is underscored by the fact that many of the population declines at the start of the chytridiomycosis panzootic occurred at pristine and protected areas.

A close relationship exists between emerging infectious disease and alien invasive species, as demonstrated by the pathways of spread for the chytridiomycosis-amphibian system (Fisher et al., 2009a). So, how can we manage the spread of such trade-associated pathogens? Invasive species can either be managed through the control of introduced species (physical, chemical, or biological control) or through the prevention of introduction (mainly through cross-boundary policies and laws). However, the strategies used in the control or prevention of invasive species do not necessarily account for the spread of infectious disease. Physical control is more effective for relatively contained invasion of conspicuous species and therefore not appropriate for pathogen control. Chemical (antifungal) or biocontrol would be more suitable for a widespread invasion such as *Bd*, but non-target mortality of amphibian hosts or other fungal species resulting from in situ application remains a high risk. Ultimately, the effective control of introduced species requires rapid response by designated authorities when an invasion is first detected, given that a country's border-level biosecurity has failed. However, unless detection is rapid, the costs of removing an invasive host species is high, and there is no guarantee that cross-transmission will not have occurred to endemic species (e.g., as was found with the transmission of *Bd* from North American bullfrogs to European *Bufo bufo*) (Garner et al., 2006). Thus, it is essential that invasion pathways are not only identified, but effectively regulated if invasion is to be prevented. Because the spread of *Bd* is associated with the amphibian trade, all intentional introductions of amphibians should be regarded as potential intentional introductions of the pathogen. In general, control of *intentional* introductions fall under the jurisdiction of governmental agencies responsible for the introductions. Unintentional introductions of *Bd* (e.g., infected frogs that escape from captivity) occur at a smaller spatial and temporal scale and are more challenging to manage. Regardless of scale, effective prevention of invasion requires a certain amount of legislation and policy development.

Another shortfall of conventional conservation is that the laws governing the conservation of biodiversity do not sufficiently deal with wildlife disease issues. International laws governing management and the conservation of biodiversity consist of agreements by cooperating countries through treaties, conventions, and voluntary participation. For instance, the Convention on Biological Diversity (CBD) consists of members who pledge to pursue a comprehensive approach to conserving biological diversity. The CBD states that all participating governments should attempt to prevent the introduction of, eradicate, or control those alien species that threaten ecosystems, habitats, or species. The conservation challenge, however, is for the individual countries that are either the source or recipient of invasive species to act on these general statements of good intent. A good example

is the *Lacey Act of 1900* that identifies a priority list of invasive species requiring conservation management. The federal agencies responsible for implementing the law are the U.S. Fish and Wildlife Service (FWS) and the National Oceanic and Atmospheric Association, but the FWS does not provide specific authority to prohibit the importation of a pathogen.

International trade in species whose existence may be threatened as a result of commerce and other trafficking is regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). However, comprehensive data covering all amphibians is hard to find because there is no global database or monitoring system for the trade in non-CITES species (Schlaepfer et al., 2005). Consequently, control over the international spread of *Bd* is not regulated under this process when the vectors are non-threatened species such as *X. laevis* and *R. catesbeiana*. The World Organisation for Animal Health (OIE) provides guidelines that can be used by member countries to protect themselves from the introduction of diseases and pathogens. Only two amphibian pathogens (*Bd* and *Ranavirus*) are currently listed as notifiable to the OIE, thus requiring regulation of the amphibian trade aimed at the prevention of disease spread. Because non-members are under no obligation to adhere to the guidelines set by the OIE and because recommendations rely on voluntary participation and lack any legally binding mechanisms, amphibians traded by both member and non-member countries are often not accompanied by veterinary certification. This shortfall in international legislation may cause ambiguity with respect to biosecurity associated with trade, ultimately contributing to the unabated global spread of pathogens.

Conservation Strategy for Amphibian Disease Threat

The contemporary approach to biological conservation is to begin with an assessment of threatened taxa, which can be used by conservationists as a source of reference for identifying species that should be prioritized for conservation research. When species are threatened by a pathogen with no apparent host specificity, however (as is the case for *Bd*), species evaluation should take on the form of a risk assessment based on biological and environmental characteristics that predispose species to disease susceptibility. Prediction models are best applied in a precautionary context, when information is used proactively to prevent a catastrophe to biodiversity (e.g., introduction of a novel pathogen). Preventing the importation of non-indigenous species in the first place is an important tool to invasive species management, but a strategy is also needed to effectively contain harmful non-indigenous species once they have become established (Schlaepfer et al., 2005).

Predict Disease Susceptibility and Emergence

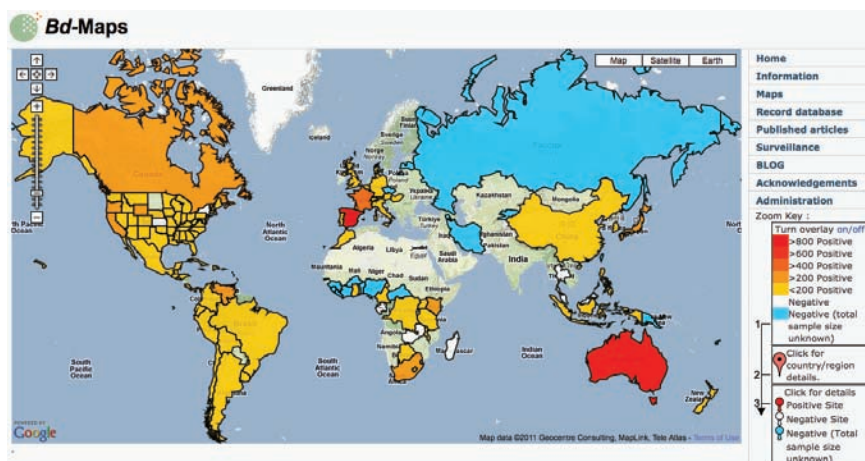
The GAA indicated disease as the third most significant threat to Anura globally, but only the seventh for salamanders, with no caecilians known to be threatened by disease (Stuart et al., 2008). When finer analyses are performed (family down to species level), the data become more appropriate as a conservation management tool. Various studies at global and regional scales have produced predictive *Bd* emergence maps and species priority lists for conservation based on multiple characteristics associated with susceptibility to *Bd* (www.bd-maps.net; Bielby et al., 2008; Rödder et al., 2009; Ron, 2005) (Figure A19-1). Some of the more common denominators collectively used in *Bd* predictive modeling include climate, altitude, conservation status, and reproductive mode of amphibian species. These integrated models can be used to predict which specific groups in any newly infected region would have a high probability of being impacted by the disease. The question of chytridiomycosis emergence is complicated by evidence that different strains of *Bd* can exhibit varying degrees of virulence (Berger et al., 2005; Fisher et al., 2009b). Therefore, the cross-regional/continental introduction of infected amphibians could introduce novel strains of *Bd* that are more virulent than those already present in a region. Consequently, there is an urgent need to abate the introduction of *Bd* into regions already affected by chytridiomycosis.

Population Management Strategies

Despite a growing wildlife disease phenomenon as human encroachment into natural areas intensifies, making ecological systems more susceptible to infection and its consequences, surprisingly little research has been done on the management of wildlife diseases (Daszak et al., 2001; Fenichel and Horan, 2007). Mendelson et al. (2006) stated that the only hope to save amphibian species from extinction is management through a combination of coordinated in situ actions with ex situ husbandry programs (e.g., survival-assurance and research colonies) on an unprecedented scale. The mandate of the IUCN is that all critically endangered and extinct in the wild taxa should be subjected to ex situ management to ensure recovery of wild populations (IUCN, 2004).

Assurance-survival populations A management strategy that results from using predictive modeling is to collect endangered species for assurance-survival populations by moving ahead of the planned spread of disease. Thus, by collecting wild animals before the arrival of disease and breeding them in captivity disease-mediated decline is prevented. Assurance-survival populations have particular application when species face threats that cannot be easily or swiftly mitigated in the wild (Pavajeau et al., 2008). The 2005 IUCN Amphibian Conservation Action Plan (ACAP) white papers state that assurance-survival populations are mandatory for amphibian species that will not persist in the wild long enough to recover naturally once environments are restored (e.g., disease is controlled)

A.



B.

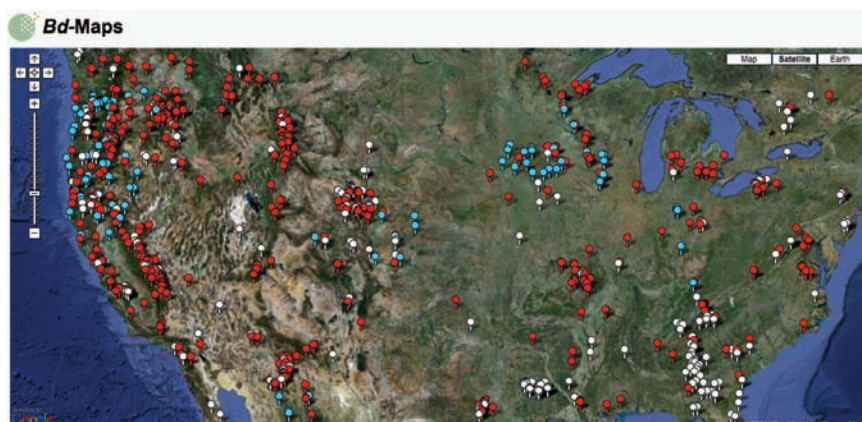


FIGURE A19-1 Maps indicating (A) the global prevalence and (B) regional U.S. prevalence of *Batrachochytrium dendrobatidis* as mapped by www.bd-maps.net.

SOURCE: The Global *Bd*-Mapping Project, www.bd-maps.net.

(IUCN, 2005). This strategy was successfully harnessed in Panama ahead of an encroaching *Bd* epidemic wave (Lips et al., 2006). Threatened, regional endemics and other specifically selected amphibian species were rescued from El Copé and El Valle and placed in the El Valle Amphibian Conservation Center, an in-country ex situ facility with the objective of long-term maintenance of species until pertinent threats could be mitigated and the species could be reintroduced (Gagliardo et al., 2008). A group of concerned conservation organizations, including the IUCN/SSC (Species Survival Commission) Conservation Breeding Specialist

Group, the World Association of Zoos and Aquariums, and the IUCN/SSC Amphibian Specialist Group initiated the Amphibian Ark (AArk), an organization that provides all levels of support to ex situ actions around the world (Pavajeau et al., 2008). Ex situ conservation centers, in addition to maintaining captive assurance populations, provide a great opportunity for much-needed research on the management of wildlife diseases.

Some concerns that have to be addressed when initiating captive assurance populations is the need of substantial resources for the construction of biosecure facilities, training of keepers, and support of amphibian husbandry requirements (Gagliardo et al., 2008; Pavajeau et al., 2008). In addition, for animals salvaged from areas with recognized disease-mediated die-offs, it is necessary to control the population-limiting infection to help ensure the success of the captive colony and to prevent introduction of disease to other collection animals (Pessier, 2008).

Disease eradication Fortunately, a variety of commonly used disinfectants, including 1 percent sodium hypochlorite (chlorine bleach) and quaternary ammonium compounds, as well as heat (60°C for 5 min) and desiccation, are effective against *Bd* (Johnson et al., 2003; Webb et al., 2007). Infected and diseased animals from zoological collections have been treated successfully with various antifungal medicines, especially itraconazole baths (Nichols et al., 2000; Parker et al., 2002; Pessier and Mendelson, 2010). Before any chemical treatment can be widely applied, its efficacy must be tested because no treatment for chytridiomycosis has proven to be consistently effective across different amphibian species and life stages (Young et al., 2007). The use of elevated environmental temperatures (37°C for 16 hours) may also clear *Bd* infection in heat-tolerant species (Woodhams et al., 2003).

However, treatment of *Bd* in wild amphibian populations presents its own challenges, and we are still far from being able to eliminate *Bd* from the environment or stop its dispersal. Although *Bd* can be killed by a range of antifungal medications, it is both impractical and ecologically dangerous to attempt such treatments in the wild due to unknown effects on ecosystems fungal components. However, mathematical models predict that mass mortalities can be reduced if infection loads in affected populations are reduced (Briggs et al., 2010). Furthermore, it is possible to eradicate a disease if the aggregate wildlife population is harvested at a specific level: below a threshold population density and below which disease prevalence declines and above which a disease becomes epidemic if a threshold exists (McCallum et al., 2001). Some of these alternative approaches have been attempted by Bosch et al. (2010) at Peñalara Natural Park in Madrid with the Common midwife toad (*Alytes obstetricans*), the Mallorcan midwife toad (*A. muletensis*), and the Betic midwife toad (*A. dickhilleni*).

Methods employed on wild-caught tadpoles included (1) thermal treatment (21°C) in captivity followed by release after metamorphosis, (2) chemical treatment with itraconazole baths in captivity and subsequent release of tadpoles into

ponds that had been desiccated during treatment, and (3) itraconazole baths in situ in artificial pools combined with habitat improvement, which included desiccation before releasing the tadpoles. The different methods resulted in varying success, although generally, released tadpoles became reinfected and metamorphs had a higher survival rate than what was determined for previous seasons. It seems that *Bd* eradication is difficult to achieve and that attempts at keeping infection levels controlled in the hope of achieving natural selection against the disease may be the only current approach for in situ mitigation (Bosch et al., 2010). In tandem with antifungal mitigations, the use of probiotic amphibian-associated bacteria holds promise for managing levels of chytrid infection in nature. Specifically, *Janthinobacterium lividum* has shown to clear infections in captive populations and is currently being widely trialed in a variety of field settings (Harris et al., 2009).

According to Schlaepfer et al. (2005), management practices that rely on continuous intervention are not sustainable indefinitely due to human and monetary resource limitations. Instead, Schlaepfer et al. (2005) suggest a novel approach where native species can respond to changes in their selective regime via evolution or learning that allows coexistence of native and introduced species in cases where the eradication of invasive species is not successful. Although this approach is suggested for invasive species that threaten local species through predation or competition, its application for a pathogen like *Bd*—capable of rapid population extirpations and even extinction through disease—remains to be explored. Some evidence exists that amphibian populations that have experienced chytridiomycosis-associated decline may partially recover and persist with endemic *Bd* infection (Retallick et al., 2004; Woodhams et al., 2007). These observations raise hope that some species can acquire resistance to chytridiomycosis in the wild, lessening the reliance on the development of survival-assurance colonies for species threatened by this disease (Mendelson et al., 2006; Young et al., 2007).

Reintroduction Ultimately, the goal of population management that begins with the capture of wild amphibians is to combine ex situ treatment with the selection of resistant individuals that could form the basis of lineages for future reintroduction. Before amphibians can be released into the wild, a number of precautions related to infectious disease need to be considered. Collections that comprise several species increase the likelihood of infection with a variety of potentially novel infectious agents (Cunningham, 1996), a situation that warrants prerelease screening for chytridiomycosis and other infectious disease, both known and unknown. Pessier (2008) demonstrated how the spectrum of infectious agents affecting amphibians is more varied than realized previously and argued for enhancement of the understanding of disease in conservation programs, which includes permanent quarantine housing of species combined with comprehensive disease surveillance and treatment. However, it is possible that hosts raised in

captivity, who are continually treated to eliminate infections, may suffer a disadvantage when released into the wild because of increased susceptibility to infection caused by relaxed selection for resistance, especially in traits that are costly to maintain (Smith et al., 2009). Given this possibility, species management programs, especially those that include captive breeding and reintroductions, may need to focus on maintaining the levels of immunity or variation in resistance that are present in natural populations (Altizer and Pedersen, 2008).

Summary

The diversity and apparent increase in wildlife diseases, including chytridiomycosis, have raised concerns that pathogens may pose a substantial threat to biodiversity. The amphibian extinction crisis has been held to represent the greatest species conservation challenge in the history of humanity (Pavajeau et al., 2008). In part, this crisis has been accelerated by the spread of *Bd* through the global amphibian trade. The ability of some amphibians to become alien invasive species while functioning as carriers of chytridiomycosis complicates conservation management of the matter. Response to amphibian threats usually involves introducing or rising protection measures of the species and/or their habitats. However, dealing with chytridiomycosis in wildlife populations requires novel strategies that conventional approaches to and policy for amphibian conservation do not adequately provide. It requires greater collaboration among wildlife ecologists, veterinarians, and conservation organizations to provide a more comprehensive mitigation strategy that may significantly reduce the risk of chytridiomycosis-mediated population declines.

The global conservation community responded to this need in the ACAP, of which the AArk plays an integral part, to select species that would otherwise go extinct for captive assurance populations until they can be secured in the wild (Pavajeau et al., 2008). Such facilities are ideally positioned to combine the management of endangered species through assurance colonies with opportunities for conservation research that may include the development of new approaches to mitigate wildlife diseases. Future efforts to better manage and protect the planet's amphibian diversity will depend on innovation from both the sciences and the world of policies and regulation. A wide spectrum of international and domestic regulation regimes already play a significant role in shaping the conservation status of amphibian biodiversity, but considerate input from the scientific community will make implementation more effective.

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A20

WHITE-NOSE SYNDROME FUNGUS (*GEOMYCES DESTRUCTANS*) IN BATS, EUROPE⁶¹

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White-nose syndrome is an emerging disease in North America that has caused substantial declines in hibernating bats. A recently identified fungus (*Geomyces destructans*) causes skin lesions that are characteristic of this disease. Typical signs of this infection were not observed in bats in North America before white-nose syndrome was detected. However, unconfirmed reports from Europe indicated white fungal growth on hibernating bats without associated deaths. To investigate these differences, hibernating bats were sampled in Germany, Switzerland, and Hungary to determine whether *G. destructans* is present in Europe. Microscopic observations, fungal culture, and genetic analyses of 43 samples from 23 bats indicated that 21 bats of 5 species in 3 countries were colonized by *G. destructans*. We hypothesize that *G. destructans* is present throughout Europe and that bats in Europe may be more immunologically or behaviorally resistant to *G. destructans* than their congeners in North America because they potentially coevolved with the fungus.

White-nose syndrome (WNS) is a recently emerged wildlife disease in North America, which in 4 years has resulted in unprecedented deaths of hibernating bats in the northeastern United States (Blehert et al., 2009; Reichard and Kunz, 2009; Turner and Reeder, 2009), and is a widespread epizootic disease among bats. Although we have searched the literature describing observations of hibernating bats, we have been unable to find any similar historical accounts of white fungus growing on live hibernating bats in North America before the recent emergence of WNS.

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In North America, WNS is known to affect 6 species of bats that use hibernation as their winter survival strategy: the big brown bat (*Eptesicus fuscus*), the eastern small-footed bat (*Myotis leibii*), the little brown bat (*M. lucifugus*), the northern long-eared bat (*M. septentrionalis*), the tricolored bat (*Perimyotis subflavus*), and the Indiana bat (*M. sodalis*) (Blehert et al., 2009; Courtin et al., 2010; Turner and Reeder, 2009). Since its detection in February 2006 in a popular tourist cave near Albany, New York, USA, WNS has spread >1,300 km into Connecticut, Massachusetts, New Hampshire, New Jersey, Pennsylvania, Tennessee, Vermont, Virginia, and West Virginia in the United States and the provinces of Ontario and Quebec in Canada (Blehert et al., 2009, Turner and Reeder, 2009; United States Geological Survey, 2010) in a pattern suggesting the spread of an infectious agent.

A recently discovered psychrophilic (cold-loving) fungus, *Geomyces destructans* (Gargas et al., 2009), has consistently been isolated from bats that meet the pathologic criteria for WNS, including colonization of skin by fungal hyphae causing characteristic epidermal erosions and ulcers that can progress to invasion of underlying connective tissue (Meteyer, et al., 2009; Reichard and Kunz, 2009). *G. destructans* is identified by its distinctive asymmetrically curved conidia and has a unique taxonomic position among other *Geomyces* spp. described to date (Gargas et al., 2009). Its closest genetic relative is *G. pannorum*, a ubiquitous psychrophilic, keratinolytic fungus that has been isolated from a variety of sources and geographic regions, including soil and the fur of wild mammals in France (Chabasse et al., 1987), floors of trains and ferryboats in Italy (Mercantini et al., 1989), boreal forests in Canada (de Bellis et al., 2007), and environmental samples from Arctic regions (Kochkina et al., 2007; Mercantini et al., 1989). *G. pannorum* var. *pannorum* has been reported as an unusual dermatophyte infecting fingernails and superficial skin of humans who have a history of close contact with soil and dust (Gianni et al., 2003; Zelenková, 2006). However, *G. destructans* differs from other common soil fungi of North America in its ability to invade the living tissues of hibernating bats.

After WNS was described in North America (Blehert et al., 2009), reports dating back to the early 1980s (Feldmann, 1984) described repeated observations of white fungal growth on muzzles of hibernating bats in Germany. However, these bats lacked the characteristics of WNS such as associated deaths. Moreover, fungus was not identified. In response to WNS in North America, researchers in Europe initiated a surveillance effort during the winter of 2008–09 for WNS-like fungal infections among hibernating populations of bats in Europe. *G. destructans* in Europe was previously reported in 1 hibernating bat that was sampled in France during March 2009 (Puechmaille et al., 2010).

In this report, we describe results of a more extensive effort by scientists from 4 countries in Europe (Germany, United Kingdom, Hungary, and Switzerland) to obtain and analyze samples from hibernating bats with white patches on their faces or wing membranes. Our objectives were to identify the fungus

colonizing such affected hibernating bats in Europe and to clarify its geographic distribution over a broad area of Europe.

Materials and Methods

During ongoing annual population surveys of caves and mines conducted by national nongovernmental organizations, hibernating bats with obvious fungal growth on their bodies (Figure A20-1, panel A) were opportunistically sampled in Germany, Switzerland, and Hungary; samples were also obtained from 2 dead bats from the same hibernaculum in the United Kingdom. Approximately 366 hibernacula were visited during mid-February–mid-April 2009: 336 in Germany, 20 in Hungary, and 10 in Switzerland. Two to 214 hibernating animals were observed at each site, with the exception of 2 sites in Germany, which harbored 2,000–7,000 animals at each site.

Samples were collected from live bats by using 2 methods. Touch imprints were obtained by holding adhesive tape against affected areas of skin or fur, or fur clippings were obtained from affected areas of bat muzzles. All species of bats in Europe are strictly protected under the Flora, Fauna, Habitat Guidelines of the European Union (92/43/ EEC) (http://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.htm) and The Agreement on the Conservation of Populations of European Bats (www.eurobats.org). We did not have permission to invasively sample or kill individual animals for histologic analysis to confirm skin infection by *G. destructans* (Meteyer et al., 2009). Samples were shipped to the Leibniz Institute of Zoo and Wildlife Research (IZW), Berlin, Germany, for further investigations.

Twenty adhesive tape samples were first screened by using light microscopy, and 21 hair samples were examined by using scanning electron microscopy for conidia characteristic of *G. destructans* (Figure A20-1, panel B). Two of the submitted samples (2 greater horseshoe bats from the United Kingdom) consisted of entire bat carcasses. Although the carcasses were examined externally for fungal growth on muzzle skin and hair, specimens were too decomposed to conduct internal pathologic examinations. Tape or hair samples from all bats were further investigated by using direct PCR amplification of fungal rRNA gene internal transcribed spacer (ITS) region DNA (ITS1, 5.8S, and ITS2). Total nucleic acids were extracted from culture, tape, or hair samples by using PrepMan Ultra Reagent (Applied Biosystems, Darmstadt, Germany) following the manufacturer's instructions.

The rRNA gene ITS region DNA was amplified by using PCR with primers ITS4 and ITS5 (White et al., 1990) and GoTaq DNA polymerase (Promega, Madison, WI, USA). Cycling parameters were an initial 2-min denaturation at 98°C; followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min; and a final extension at 72°C for 7 min. For fungal isolates, rRNA gene small subunit (SSU) DNA was amplified by us-

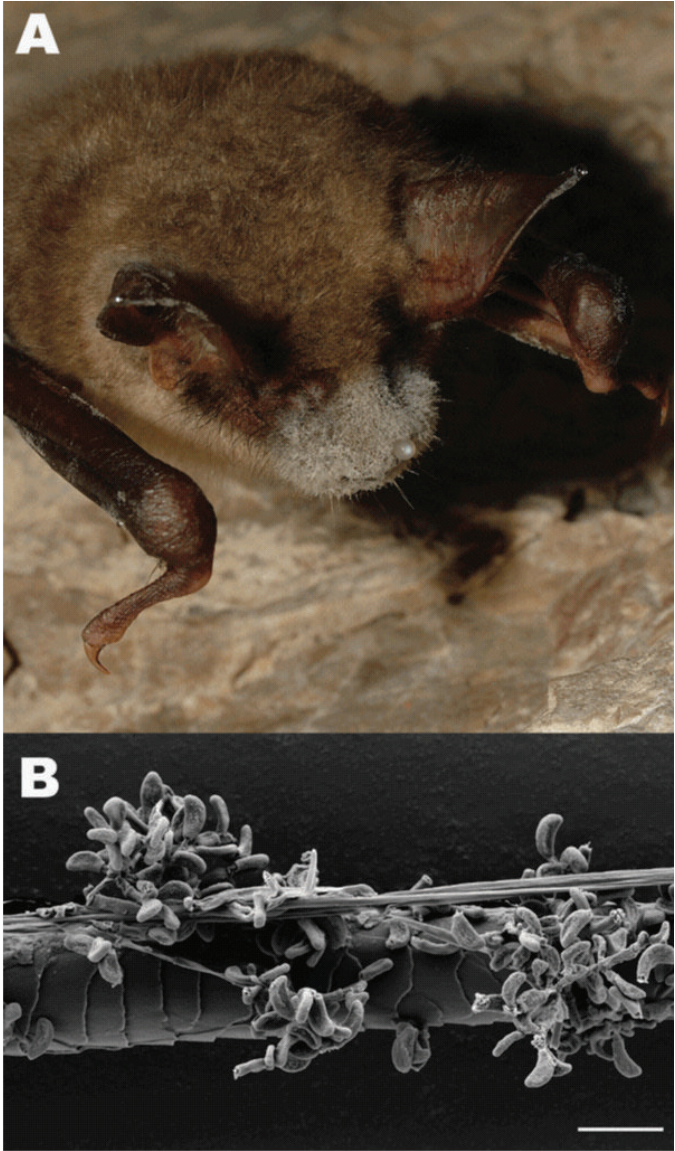


FIGURE A20-1 (A) Greater mouse-eared bat (*Myotis myotis*) with white fungal growth around its muzzle, ears, and wing membranes (photograph provided by Tamás Görföl). (B) Scanning electron micrograph of a bat hair colonized by *Geomyces destructans*. Scale bar = 10 μm .

ing PCR with primers nu-SSU-0021–5′ (White et al., 1990) and nu-SSU-1750–3′ (Gargas and Taylor, 1992) as above, except the extension time was increased to 2 min. Sequencing primers were PCR primers; nu-SSU-0402–5′ (Gargas and Taylor, 1992), nu-SSU-1150–5′ (White et al., 1990), nu-SSU-0497–3′ (Gargas and Taylor, 1992), and nu-SSU-1184–3′ (Gargas et al., 1995) were added for SSU. PCR products were sent to the Robert Koch Institute, Berlin, Germany, for direct sequencing.

Culture analyses of samples were performed at Munich University Hospital and IZW. After examining tape impressions by using light microscopy, we identified small areas with fungal conidia characteristic of *G. destructans* and excised them with a sterile scalpel blade. Half of the excised material was used for PCR; the remaining sample and samples of individual hairs with microscopic indication of *G. destructans* were immediately placed onto Sabouraud dextrose agar plates containing gentamicin and chloramphenicol and incubated at 4°C and 8°C. *G. destructans* isolates obtained during this study are maintained at IZW.

Results

We obtained and analyzed samples from live bats with obvious fungal growth on their bodies found between mid-February and the end of March 2009 at 11 sites (8 in Germany, 1 in Hungary, and 2 in Switzerland). Samples were also obtained from an additional bat in Germany in February 2008 and from 2 dead bats from a site in the United Kingdom in March 2009 (Tables A20-1, A20-2). All 12 hibernacula sampled contained 1–5 animals that exhibited obvious fungal growth. Forty-three samples were obtained from these 12 hibernacula and represented 23 adult bats of 6 species: 1 Brandt bat (*M. brandtii*), 3 pond bats (*M. dasycneme*), 1 Daubenton bat (*M. daubentonii*), 1 lesser mouse-eared bat (*M. oxygnathus*), 15 greater mouse-eared bats (*M. myotis*), and 2 greater horseshoe bats (*Rhinolophus ferrumequinum*).

After direct PCR amplification and DNA sequence analysis of fungal rRNA gene ITS regions, genetic signatures 100% identical with those from *G. destructans* type isolate NWHC 20631–21 (GenBank accession no. EU884921) were identified from 21 of 23 bats examined: 15/15 from Germany, 2/2 from Hungary, and 4/4 from Switzerland. Both bats from the United Kingdom were colonized by *Penicillium* sp. (Tables A20-1, A20-2). Fungi with conidia morphologically identical to those of *G. destructans* (Figure A20-1, panel B) as described by Gargas et al. (2009) were isolated in axenic cultures from 8 of 23 bats examined: 3/15 from Germany, 1/2 from Hungary, and 4/4 from Switzerland (Tables A20-1, A20-2; Figure A20-2).

Consistent with published descriptions for *G. destructans* (Gargas et al., 2009), fungal colonies grew slowly and within 14 days attained diameters of 1.0 mm at 4°C and 4.0–5.0 mm at 8°C; no growth occurred at 25°C. The sensitivity of our method for isolating *G. destructans* from bat hair was comparable to

TABLE A20-1 Bats Tested for *Geomyces destructans* by Using Microscopy, Fungal Culture, or PCR Analysis, by Country, Europe*

Species (common name)	No. positive/no. tested			
	Germany	Switzerland	Hungary	United Kingdom
<i>Myotis myotis</i> (greater mouse-eared bat)	10/10	4/4	1/1	-
<i>M. dasycneme</i> (pond bat)	3/3	-	-	-
<i>M. daubentonii</i> (Daubenton bat)	1/1	-	-	-
<i>M. brandtii</i> (Brandt bat)	1/1	-	-	-
<i>M. oxygnathus</i> (lesser mouse-eared bat)	-	-	1/1	-
<i>Rhinolophus ferrumequinum</i> (greater horseshoe bat)	-	-	-	0/2

*-, species not obtained in this country.

published diagnostic sensitivity for culturing *G. destructans* from bat skin (Lorch et al., 2010). Subsequent PCR/DNA sequencing analyses of the 8 isolates indicated that they all had rRNA gene ITS and SSU region DNA sequences identical to those of *G. destructans* type isolate NWHC 20631–21 (GenBank accession nos. EU884921 for ITS and FJ231098 for SSU).

Unlike other bats sampled in this study, the 2 greater horseshoe bats from the United Kingdom were found dead, and their nostrils were colonized by *Penicillium* sp. These bats did not fulfill the pathologic criteria for WNS (Meteyer, et al., 2009) because fungal hyphae did not invade the epidermis but remained within the superficial layer of the epidermal stratum corneum. A more complete description of the postmortem analysis of the greater horseshoe bats has been reported (Barlow et al., 2009). *G. destructans* was not isolated in culture, and its genetic signature was not identified by PCR and DNA sequencing of samples collected from greater horseshoe bats.

Discussion

Laboratory analyses demonstrated that 5 species of the genus *Myotis* in Europe harbored *G. destructans*; male and female bats were equally affected. Despite laboratory confirmation that bats obtained in Germany, Switzerland, and Hungary were colonized by *G. destructans*, deaths were not observed at collection sites. Puechmaille et al. (2010) reported a similar observation with a greater mouse-eared bat in France. Additionally, a lesser mouse-eared bat from Hungary with visible fungal infection during hibernation, from which *G. destructans* was isolated, was recaptured 5 months later (August 2009) and showed no external signs of fungal infection. On February 19, 2010, the same bat was again observed in the same hibernaculum without any visible sign of fungal growth. However, 7 other bats within that group of 55 animals displayed obvious fungal growth but were not sampled for this study.

TABLE A20-2 Fungal Culture and PCR Results for 23 Bats with Evidence of Fungal Colonization Tested by Light or Electron Microscopy, Europe*

Country/location no.†	Sample source	Species	Collection date	No./ hibernacula	PCR result	Culture result	GenBank accession no.	
							ITS	SSU rRNA
Germany/4	Hair 2	<i>Myotis dasycneme</i>	2008 Feb 25	10	+	+	GU350437	GU350442
Germany/8	Hair 7	<i>M. myotis</i>	2009 Mar 3	214	+	+	GU350436	GU350441
Germany/7	Tape 8	<i>M. myotis</i>	2009 Mar 7	57	+	+	GU999986	GU999983
Hungary/9	Hair 16	<i>M. myotis</i>	2009 Mar 29	64	+	+	GU350434	GU350439
Switzerland/10	Tape 10	<i>M. myotis</i>	2009 Apr 5	25	+	+	GU350433	GU350438
Switzerland/10	Tape 11	<i>M. myotis</i>	2009 Apr 5	25	+	+	GU999984	GU999981
Switzerland/11	Tape 12	<i>M. myotis</i>	2009 Apr 5	25	+	+	GU999985	GU999982
Switzerland/10	Tape 20	<i>M. myotis</i>	2009 Apr 11	25	+	+	GU350435	GU350440
Germany/1	Hair 1	<i>M. myotis</i>	2009 Feb 21	65	+	–	HM222616	–
Germany/6	Hair 20	<i>M. myotis</i>	2009 Mar 13	100	+	–	HM222617	–
Germany/2	Tape 1	<i>M. myotis</i>	2009 Feb 26	≈2,000	+	–	HM222618	–
Germany/2	Tape 2	<i>M. myotis</i>	2009 Feb 26	≈2,000	+	–	HM222619	–
Germany/8	Tape 5	<i>M. myotis</i>	2009 Mar 3	214	+	–	HM222620	–
Germany/8	Tape 6	<i>M. myotis</i>	2009 Mar 3	214	+	–	HM222621	–
Germany/7	Tape 9	<i>M. myotis</i>	2009 Mar 7	57	+	–	HM222622	–
Germany/6	Tape 16	<i>M. myotis</i>	2009 Mar 13	100	+	–	HM222623	–
Germany/8	Hair 6	<i>M. brandtii</i>	2009 Mar 3	214	+	–	HM222624	–
Germany/5	Hair 3	<i>M. dasycneme</i>	2009 Feb 28	29	+	–	HM222625	–
Germany/6	Tape 17	<i>M. dasycneme</i>	2009 Mar 13	100	+	–	HM222626	–
Germany/3	Hair 17	<i>M. daubentonii</i>	2009 Mar 5	≈7,000	+	–	HM222627	–
Hungary/9	Tape 13	<i>M. oxygnathus</i>	2009 Mar 29	64	+	–	HM222628	–
United Kingdom/12	Hair 10	<i>Rhinolophus ferrumequinum</i>	2009 Mar 11	558	–‡	–‡	HM222629	–
United Kingdom/12	Hair 11	<i>R. ferrumequinum</i>	2009 Mar 11	558	–‡	–‡	HM222630	–

*ITS, internal transcribed spacer; SSU, small subunit; tape, touch imprint with adhesive tape.

†Location number corresponds to a hibernation site in Figure A20-2.

‡Although samples were negative for *G. destructans*, they were positive for *Penicillium* sp. by PCR and culture.

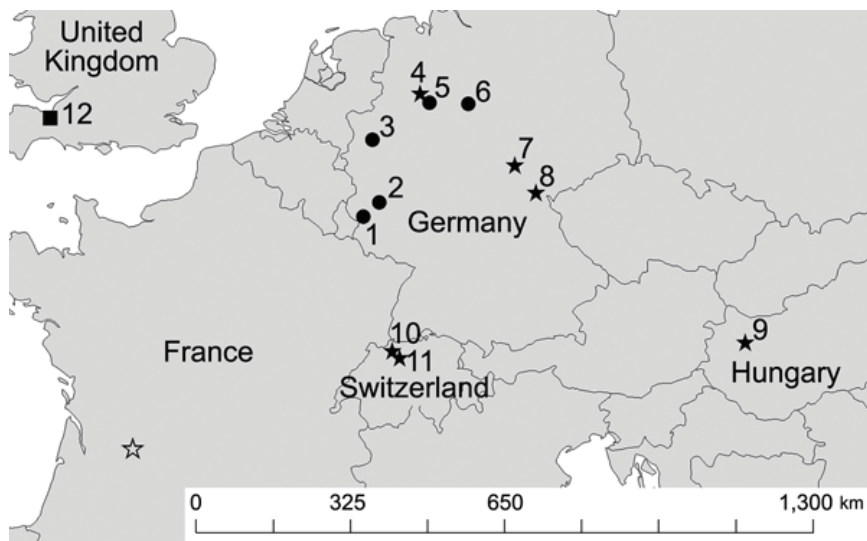


FIGURE A20-2 Locations in Europe of bats positive for *Geomyces destructans* by PCR alone (circles) or by PCR and culture (solid stars) and bats negative for *G. destructans* but positive for other fungi (square). Numbers for locations correspond to those in Table A20-2. Sites 7, 8, and 9 had additional bats that were positive for *G. destructans* only by PCR. Location of a bat positive for *G. destructans* in France (Puechmaille et al., 2010) is indicated by an open star. Some sites had >1 bat species with evidence of colonization by *G. destructans*.

In contrast, decreases in hibernating bat colonies infected by *G. destructans* in North America are often >90% (Reichard and Kunz, 2009; Turner and Reeder, 2009), and mortality rates similar in magnitude would be difficult to miss among closely monitored winter populations of bats in Europe. Biologists in Germany and Switzerland have conducted annual censuses of bat hibernacula since the 1930s and 1950s, respectively. In Hungary, the largest hibernacula have been annually monitored since 1990. Similar death rates to those caused by WNS in hibernating bats in North America have never been documented in countries in Europe in which *G. destructans* has now been identified.

Although distribution of *G. destructans* in bats across Europe has not been exhaustively characterized, opportunistic sampling conducted as part of this study during the winter of 2008–09 demonstrated that the fungus was present on bats in 3 countries (Figure A20-2). The 2 most distant points from which bats colonized with *G. destructans* have been identified were separated by >1,300 km. Despite the observed distribution of *G. destructans* in Europe (Figure A20-2), the 5 bat species from which *G. destructans* was detected migrate average distances <100 km between their summer and winter roosting sites (Hutterer et al., 2005),

indicating that the fungus is most likely spread as local bat populations emerge from hibernacula, disperse, and interact with populations within their dispersal range. Identification of bats colonized by *G. destructans* from such distant sites, in addition to the relatively homogenous distribution of the fungus among sites in Germany, suggests that *G. destructans* may be widespread in Europe.

Regardless of widespread occurrence of *G. destructans* among bat species in Europe (Figure A20-2), deaths of bats in Europe caused by WNS, similar to those caused by WNS in North America, have not been observed. Although no bat species migrates between Europe and North America or is present on both continents (Dietz et al., 2009; Nowak, 1999), many species of the genus *Myotis* are infected by *G. destructans* on each continent. Although the mechanism(s) by which hibernating bats died because of infection with *G. destructans* in North America is not yet understood, bat species in Europe may exhibit greater resistance or respond differently to infection by this fungus than their counterparts in North America.

Before the emergence of WNS in North America, large aggregations of hibernating bats ranging from 1,000 to 50,000 animals were common in caves and mines of affected regions, and many hibernation sites in regions of North America still unaffected by WNS contained tens of thousands of bats during winter (some contain hundreds of thousands) (Barbour and Davis, 1969). In contrast, aggregations of bats hibernating in caves and mines in Europe rarely exceed 1,000 animals. However, larger hibernating groups have been observed at a few natural sites, such as a cave in northern Germany with 13,000–18,000 bats (Petermann and Boye, 2006) and human-made structures (e.g., Daubenton bats in bunkers and catacombs) (Dietz et al., 2009). If host density plays a role in *G. destructans* transmission or deaths of bats, such as through increased disturbance of clustered bats, the bats in Europe may experience lower mortality rates because they form smaller hibernation groups composed of small clusters or individual bats. Apparent continental differences in susceptibility of hibernating bats to deaths associated with skin infection by *G. destructans* may indicate either circumstantial or evolved resistance in bats in Europe.

G. destructans has been detected in North America only in states and provinces where WNS has also been observed and in contiguous states. Recent emergence and spread of *G. destructans* with associated deaths of bats throughout hibernacula in the northeastern United States (Turner and Reeder, 2009) may suggest ecologic release of an exotic pathogen into an uninfected ecosystem. Although this suggestion remains a hypothesis and how *G. destructans* may have been introduced to the United States is not known, initial documentation of WNS in a popular tourist cave near Albany, New York (Blehert et al., 2009), suggests that a human vector could have been involved.

There are many examples of unintended introductions of fungal pathogens, particularly of those affecting plants and ectothermic animals with tissue temperatures permissive to fungal infection (Casadevall, 2005; Desprez-Loustau et al.,

2007; Robert and Casadevall, 2009). One case with striking similarities is the panzootic chytrid fungus (*Batrachochytrium dendrobatidis*), which has caused global decreases among amphibian species (Fisher et al., 2009). As with skin infection by *B. dendrobatidis* in amphibians, which can alter body electrolyte levels and lead to cardiac arrest (Voyles et al., 2009), skin infection by *G. destructans* in hibernating bats may also kill by causing irreversible homeostatic imbalance because wing membranes play major roles in water balance, circulation, and thermoregulation of hibernating bats during winter (Davis, 1970; Makanya and Mortola, 2007).

Bat species in Europe may be immunologically or behaviorally resistant to *G. destructans* because of having coevolved with the fungus. Additionally, microbial flora of bat skin or other abiotic surfaces in bat hibernacula in Europe may have also coevolved to incorporate *G. destructans* as a nonpathogenic component of the microbial community. Conversely, possible recent introduction of *G. destructans* into the United States, with subsequent infection of bat species in North America and ecosystems not infected with the fungus, provides a potential explanation for the devastating effects of WNS in North America. Although bats are reservoirs of various pathogens (Calisher et al., 2006; Wibbelt et al., 2009), research into the immune function of bats, particularly during hibernation, is just beginning.

In conclusion, nondetrimental colonization of bat species in Europe by *G. destructans* may be relatively common (Figure A20-2), and historical reports (Feldmann, 1984) suggest that such colonization of hibernating bats in Europe has occurred for several decades. In contrast to recent mass deaths associated with *G. destructans* skin infection, which is the hallmark of WNS in North America, bats in Europe appear to coexist with *G. destructans*. Studies to investigate mechanisms of pathogenesis, microbial ecology, and phylogeography of *G. destructans* will be essential for developing a comprehensive understanding of WNS. In particular, testing the hypotheses that bats in Europe are more resistant to fungal skin infection by *G. destructans*, that *G. destructans* was introduced from Europe to North America, and that environmental circumstances limit the pathogenicity of *G. destructans* in Europe seem warranted. Divergent manifestations of infection by *G. destructans* in bats in Europe and North America provide a unique opportunity to address these research objectives with the ultimate goals of better understanding WNS and developing sound strategies to manage the devastating effects of this emerging wildlife disease in North America.

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A21

**PAN-EUROPEAN DISTRIBUTION OF WHITE-NOSE
SYNDROME FUNGUS (*GEOMYCES DESTRUCTANS*)
NOT ASSOCIATED WITH MASS MORTALITY***

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Abstract

Background:

The dramatic mass mortalities amongst hibernating bats in Northeastern America caused by “white nose syndrome”(WNS) continue to threaten populations of different bat species. The cold-loving fungus, *Geomyces destructans*, is the most likely causative agent leading to extensive destruction of the skin, particularly the wing membranes. Recent investigations in Europe confirmed the presence of the fungus *G. destructans* without associated mass mortality in hibernating bats in six countries but its distribution remains poorly known.

Methodology/Principal Findings:

We collected data on the presence of bats with white fungal growth in 12 countries in Europe between 2003 and 2010 and conducted morphological and genetic analysis to confirm the identity of the fungus as *Geomyces destructans*. Our results demonstrate the presence of the fungus in eight countries spanning over 2000 km from West to East and provide compelling photographic evidence for its presence in another four countries including Romania, and Turkey. Furthermore, matching prevalence data of a hibernaculum monitored over two consecutive years with data from across Europe show that the temporal occurrence of the fungus, which first becomes visible around February, peaks in March but can still be seen in some torpid bats in May or June, is strikingly similar throughout Europe. Finally, we isolated and cultured *G. destructans* from a cave wall adjacent to a bat with fungal growth.

Conclusions/Significance:

G. destructans is widely found over large areas of the European continent without associated mass mortalities in bats, suggesting that the fungus is native to Europe. The characterisation of the temporal variation in *G. destructans* growth on bats provides reference data for studying the spatio-temporal dynamic of the fungus. Finally, the presence of *G. destructans* spores on cave walls suggests that hibernacula could act as passive vectors and/or reservoirs for *G. destructans* and therefore, might play an important role in the transmission process.

Introduction

White nose-syndrome (WNS) is a devastating disease causing mass mortalities in hibernating bats in North-America. In May 2009, it was estimated that over one million bats had died from the disease which was first documented in February 2006 at Howe’s Cave, West of Albany, New York (Anonymous, 2009). A visually conspicuous white fungus grows on the face, ears, or wings of

stricken bats with hyphae penetrating deep into the connective tissue of glabrous skin and snout (Meteyer et al., 2009) and causing severe damage (Reichard and Kunz, 2009). The fungus associated with WNS is a newly described, psychrophilic (cold-loving) species (*Geomyces destructans*) (Gargas et al., 2009), closely related to other psychrophilic species of *Geomyces* (Rice and Currah, 2006; Puechmaille et al., 2010). Although it is not yet conclusively proven whether *G. destructans* is the causative agent of the disease or if other co-factors are necessary for disease to occur, the fungus is always found on bats at WNS sites where hibernating bats experience mass mortalities (Blehert et al., 2009). To date, bacteriological, virological, parasitological and pathological evaluations as well as studies of toxic contaminants have not identified the consistent presence of any other agents/cause of death. The lack of evidence for the involvement of other agents or compounds reinforces the suspicion that *G. destructans* is the causative agent of WNS mortality (Meteyer et al., 2009; Blehert et al., 2009; Kannan et al., 2010; Courtin et al., 2010).

Geomyces destructans has been found in nine species of bats in North-America, from the provinces of Ontario and Quebec in Canada south and west to the states of Tennessee and Oklahoma in the USA (Anonymous, 2010). Three recent studies investigating samples collected in 2008–2010 have shown that *G. destructans* was also present in six European countries (France, Germany, Switzerland, Czech Republic, Slovakia & Hungary) (Puechmaille et al., 2010; Martínková et al., 2010; Wibbelt et al., 2010). Nevertheless, the geographic coverage of these studies was limited and the extent of the distribution of *G. destructans* in Europe remains poorly known. In this paper, we combine previously published data on the distribution of *G. destructans* in Europe (Puechmaille et al., 2010; Martínková et al., 2010; Wibbelt et al., 2010) with new data from twelve countries covering 2,400 km from West to East (France to Turkey) and 1,900 km from North to South (Estonia to Turkey) to demonstrate the widespread presence of *G. destructans* on multiple species of hibernating bats in Europe without associated mass mortality.

Results

Review of data on Geomyces destructans in European bats, 2008–2010

Although photographs of bats with fungal growth similar to *G. destructans* were published in Germany in the 1980's (Feldmann, 1825), and also taken in the 1990's in the Czech Republic (Martínková et al., 2010), there have been no confirmed records of *G. destructans* in Europe prior to 2008 (Puechmaille et al., 2010; Wibbelt et al., 2010). In 2010, *G. destructans* has been confirmed by morphological and genetic analyses from samples collected during the winters 2007/2008, 2008/2009 and 2009/2010 in six European countries (Puechmaille et al., 2010; Martínková et al., 2010; Wibbelt et al., 2010). In France, Hungary,

Switzerland and Slovakia, the fungus has been confirmed from 1–2 location(s) per country, whereas it has been confirmed at 8 sites in Germany and 23 sites in the Czech Republic (Puechmaille et al., 2010; Martínková et al., 2010; Wibbelt et al., 2010). All confirmed detections of *G. destructans* in Europe have been made by isolating and/or genetically identifying the fungus from hairs, swabs or touch imprints from bats (Puechmaille et al., 2010; Martínková et al., 2010; Wibbelt et al., 2010). In Europe, eight species of *Myotis* have been observed being colonised by *G. destructans*: *M. Myotis*, *M. blythii* (referred to as *M. oxygnathus* in [Wibbelt et al., 2010]), *M. mystacinus*, *M. daubentonii*, *M. dasycneme*, *M. nattereri*, *M. bechsteinii* and *M. brandtii*. Species from other bat families were present in the caves with infected individuals (e.g., *Miniopteridae*: *Miniopterus schreibersii*; *Rhinolophidae*: *Rhinolophus hipposideros* and *R. ferrumequinum*), but no *G. destructans* has been confirmed from these species. Previous extensive surveys of cave fungi in Europe (i.e., Nováková, 2009; Mosca and Campanino, 1962; Bastian et al., 2009) or fungi associated with insects hibernating in underground sites (Kubátová and Dvořák, 2005) never reported *G. destructans* in their inventory, although some other species of *Geomyces* were recovered (Nováková, 2009; Mosca and Campanino, 1962; Bastian et al., 2009).

New data on G. destructans in Europe 2003–2010

During winter hibernation counts, a total of 107 bats from 56 sites in twelve European countries were reported to have visible white fungal growth (Tables A21-1, A21-2, A21-3 and Figure A21-1). This represents the first records from nine countries (Austria, Belgium, Denmark, Estonia, The Netherlands, Poland, Romania, Turkey and Ukraine). One hundred and five bats were alive and two of them were found dead in hibernacula. These 107 bats belonged to eight different species of *Myotis*: *M. Myotis* (59), *M. dasycneme* (26), *M. mystacinus* (9), *M. daubentonii* (4), *M. Myotis/blythii* (3), *M. blythii* (3), *M. nattereri* (1), *M. escalerae/sp. A* (1) and *M. brandtii* (1). Of these, molecular and morphological identifications of the colonising fungus were carried out in 23 cases (Table A21-1), while only photographic evidence was obtained for a further 50 cases (Table A21-2 and Figure A21-2). The remaining 34 cases were based on reports of visual observations of a white fungal growth on bat snouts and/or ears (Table A21-3), which was very similar to pictures presented in Figure A21-2. All 84 bats reported in Tables A21-2 and A21-3 are considered as *Gd*-suspects (bats showing fungal growth that is thought to be *G. destructans*).

Geomyces destructans identification

Out of a total of 107 bats with fungal growth, 22 were sampled, 16 with touch imprints and 6 with cotton swabs. The 22 bats sampled (20 alive and 2 dead) belonged to the species of *Myotis* from which *G. destructans* had been pre-

TABLE A21-1 Confirmed Records of *Geomyces destructans* on Hibernating Bats in Europe and Details of the Culture and Genetic Analyses

Country	Lat	Lon	Date	Host species	Culture	PCR	GenBank No.
France*	49.9	4.1	04/03/2010	<i>Myotis mystacinus</i>	GuH-04032010	- [‡]	n/a
France*	50.6	2.5	22/02/2010	<i>Myotis nattereri</i>	ThC-22022010	- [‡]	n/a
France [†]	47.7	-2.1	04/03/2010	<i>Myotis myotis</i>	Mmyo-FR-1	+	JF502415
Belgium	49.8	5.3	03/04/2010	<i>Myotis myotis</i>	Mmyo-BE-1	+	JF502414
Belgium [†]	50.8	5.6	18/03/2010	<i>Myotis mystacinus</i>	Mmys-BE-1	+	JF502407
Belgium [†]	50.8	5.6	18/03/2010	<i>Myotis mystacinus</i>	n/a	+	n/a
Netherlands [†]	52.0	5.8	09/03/2010	<i>Myotis daubentonii</i>	Mdau-NL-1	+	JF502411
Netherlands	52.1	4.3	27/02/2010	<i>Myotis dasycneme</i>	Mdas-NL-1	+	JF502410
Germany [†]	49.7	7.4	10/03/2010	<i>Myotis myotis</i>	Mmyo-DE-12	+	JF502401
Germany	49.8	9.6	22/03/2010	<i>Myotis myotis</i>	Mmyo-DE-14	+	JF502403
Germany	50.7	13.7	20/03/2010	<i>Myotis myotis</i>	Mmyo-DE-13	+	JF502402
Germany	50.9	7.5	18/04/2009	<i>Myotis myotis</i>	Mmyo-DE-10	+	JF502399
Germany	51.2	8.1	21/03/2010	<i>Myotis mystacinus</i>	Mmys-DE-2	+	JF502409
Germany	51.2	8.1	21/03/2010	<i>Myotis mystacinus</i>	Mmys-DE-3	+	n/a
Germany	51.2	8.1	07/03/2010	<i>Myotis myotis</i>	Mmyo-DE-11	+	JF502400
Germany	51.2	8.1	07/03/2010	<i>Myotis myotis</i>	Mmyo-DE-16	+	n/a
Germany [†]	52.3	9.5	23/03/2010	<i>Myotis myotis</i>	Mmyo-DE-15	+	JF502404
Germany [†]	52.3	9.4	23/03/2010	<i>Myotis mystacinus</i>	Mmys-DE-1	+	JF502408
Hungary	47.1	17.6	24/03/2010	<i>Myotis myotis</i>	Mmyo-HU-2	+	JF502405
Hungary	47.1	17.6	24/03/2010	<i>Myotis myotis</i>	Mmyo-HU-3	+	n/a
Poland	50.8	16.7	07/03/2010	<i>Myotis myotis</i>	Mmyo-PL-1	+	JF502413
Estonia ^{#,†}	59.3	24.6	01/06/2010	<i>Myotis brandtii</i>	EsT-01062010	+	JF502412
Ukraine	48.7	26.6	17/03/2010	<i>Myotis myotis</i>	Mmyo-UA-1	+	JF502406

*Dead bat.

[‡]Environmental sample (individual observed 23/05/2010; see text for further explanations).[†]Photograph of the bat shown in Figure A21-2.[#]Samples were negative for *G. destructans* but amplified another fungus. doi:10.1371/journal.pone.0019167.t001

viously isolated (see list above). In some cases, we were not able to discriminate between *M. Myotis* and *M. blythii* (referred to as *M. Myotis/blythii*) as well as between the newly recognised *M. escalerae* (Ibañez et al., 2006; Cabrera, 1904) and *Myotis* sp. A (Garcia-Mudarra et al., 2009), a yet undescribed cryptic species from the *M. nattereri* species complex (Ibañez et al., 2006; Mayer et al., 2007). Additionally, swab samples were collected from the tunnel wall of an Estonian hibernaculum. On the 23rd May 2010, a *M. brandtii* was observed in this hibernaculum with white fungal growth on its snout (Figure A21-2A) but no sample was collected at the time. When the site was revisited for sample collection on the 1st of June 2010, the bat had left the site so samples were collected by swabbing the wall of the tunnel where the bat was seen nine days before. Four cotton swabs were used to sample different areas a few centimetres around the location where

TABLE A21-2 Suspected Photographic Records of *Geomyces destructans* on Hibernating Bats in Europe.

Country	Lat.	Lon.	Date	Host species	No. Individual
France	44.8	1.6	25/04/2008	<i>Myotis myotis</i>	1
France [†]	42.6	2.2	26/06/2010	<i>Myotis escaleraei</i> /sp.A	1
France	47.7	22.1	04/03/2010	<i>Myotis myotis</i>	1
France [†]	45.0	2.0	13/02/2010	<i>Myotis myotis</i>	2
France	47.3	6.2	04/03/2010	<i>Myotis myotis</i>	3
France	50.4	3.5	01/03/2008	<i>Myotis mystacinus</i>	1
France	47.2	1.4	24/02/2010	<i>Myotis myotis</i>	2
Belgium	50.8	5.6	09/02/2008	<i>Myotis dasycneme</i>	1
Belgium	50.8	5.6	20/03/2008	<i>Myotis daubentonii</i>	1
Belgium	50.8	5.6	17/01/2010	<i>Myotis dasycneme</i>	1
Belgium [†]	50.3	5.9	07/03/2010	<i>Myotis myotis</i>	1
Belgium	50.8	5.7	13/03/2010	<i>Myotis dasycneme</i>	1
Netherlands	52.1	4.3	26/03/2008	<i>Myotis dasycneme</i>	1
Netherlands	52.1	4.3	18/02/2008	<i>Myotis dasycneme</i>	1
Netherlands	52.0	5.7	04/03/2010	<i>Myotis mystacinus</i>	1
Denmark [†]	56.4	9.1	14/03/2010	<i>Myotis dasycneme</i>	2
Germany	51.8	10.8	02/02/2008	<i>Myotis myotis</i>	1
Germany	51.6	10.5	07/02/2010	<i>Myotis myotis</i>	1
Germany	51.7	10.3	20/03/2010	<i>Myotis myotis</i>	1
Germany [†]	52.3	9.5	23/03/2010	<i>Myotis mystacinus</i>	1
Germany	52.3	9.5	23/03/2010	<i>Myotis dasycneme</i>	1
Germany	52.1	8.2	21/03/2007	<i>Myotis daubentonii</i>	1
Germany	52.1	8.2	14/03/2007	<i>Myotis dasycneme</i>	1
Germany	52.2	8.0	04/02/2008	<i>Myotis myotis</i>	1
Austria [†]	46.8	16.0	07/02/2007	<i>Myotis myotis</i>	1
Hungary	47.1	17.6	24/02/2007	<i>Myotis myotis</i>	1
Hungary	47.1	17.6	23/02/2009	<i>Myotis myotis</i>	1
Hungary	46.2	18.1	03/03/2009	<i>Myotis myotis/blythii</i>	1
Hungary	48.5	20.5	18/02/2010	<i>Myotis blythii</i>	1
Hungary	47.1	17.6	19/02/2010	<i>Myotis blythii</i>	1
Hungary [†]	47.1	17.6	19/02/2010	<i>Myotis myotis</i>	2
Poland [†]	50.8	16.7	07/03/2010	<i>Myotis myotis</i>	1
Ukraine [†]	48.8	26.6	13/02/2010	<i>Myotis myotis</i>	1
Ukraine	48.8	26.6	17/03/2010	<i>Myotis myotis</i>	8
Romania [†]	46.8	22.6	29/03/2008	<i>Myotis blythii</i>	1
Romania	45.4	25.2	14/03/2009	<i>Myotis myotis/blythii</i>	1
Turkey [†]	41.9	27.9	22/03/2009	<i>Myotis myotis/blythii</i>	1

[†]Photograph of the bat shown in Figure A21-2. doi:10.1371/journal.pone.0019167.t002

the bat was observed. The four swabs were then streaked onto four Sabouraud's agar plates each and monitored regularly to physically remove any fungal growth that was not similar to *G. destructans*. Although the amount of fungi varied per swab sample, *G. destructans* was recovered from all four swabs, henceforth considered as a single sample, bringing the total of samples analysed to 23. No mass mortality was reported at any of the sites investigated.

TABLE A21-3 Suspected Visual Records of *Geomyces destructans* on Hibernating Bats in Europe

Country	Lat.	Lon.	Date	Host species	No. Individual
France	49.1	6.6	06/04/2009	<i>Myotis myotis</i>	1
France	48.5	6.9	28/02/2009	<i>Myotis myotis</i>	1
France	48.3	7.1	29/03/2009	<i>Myotis myotis</i>	1
France	48.3	5.7	16/03/2008	<i>Myotis myotis</i>	1
France	47.9	6.8	03/03/2010	<i>Myotis myotis</i>	2
France	49.5	5.2	04/03/2010	<i>Myotis myotis</i>	1
France	48.9	0.3	06/02/2010	<i>Myotis myotis</i>	1
France	47.2	5.7	20/02/2010	<i>Myotis myotis</i>	3
Netherlands	52.1	4.3	10/03/2005	<i>Myotis dasycneme</i>	2
Netherlands	52.1	4.3	24/06/2006	<i>Myotis dasycneme</i>	1
Netherlands	52.1	4.3	07/03/2007	<i>Myotis dasycneme</i>	1
Netherlands	52.1	4.3	15/03/2008	<i>Myotis dasycneme</i>	3
Netherlands	52.1	4.3	30/03/2008	<i>Myotis dasycneme</i>	2
Netherlands	52.1	4.3	05/04/2008	<i>Myotis dasycneme</i>	1
Netherlands	52.1	4.3	12/04/2008	<i>Myotis dasycneme</i>	1
Netherlands	52.1	4.3	13/02/2004	<i>Myotis dasycneme</i>	2
Netherlands	52.1	4.3	05/04/2003	<i>Myotis dasycneme</i>	1
Netherlands	52.1	4.3	26/03/2008	<i>Myotis dasycneme</i>	1
Netherlands	52.1	4.3	10/03/2005	<i>Myotis dasycneme</i>	1
Germany	50.9	13.3	23/03/2010	<i>Myotis daubentonii</i>	1
Germany	49.9	7.4	14/03/2010	<i>Myotis Myotis</i>	1
Ukraine	48.8	26.6	17/03/2010	<i>Myotis Myotis</i>	4
Romania	47.0	22.4	08/04/2008	<i>Myotis Myotis</i>	1

doi:10.1371/journal.pone.0019167.t003

Out of 23 samples investigated in the laboratory, 14 of the 16 touch imprint samples presented characteristic conidia when observed under a microscope and two of them were doubtful; none of the cotton swabs were inspected under a microscope prior to culture. Cultures from 22 of these samples were successful. The two dead bats investigated did not reveal the presence of *G. destructans* but other fungal species such as *Mucor* sp. and *Cladosporium* sp. were identified (data not shown).

DNA was isolated from the 22 cultures of which 20 showed morphological similarity to *G. destructans* (e.g., curved conidia) and from one touch imprint with unsuccessful culture attempts. Amplification and sequencing of the internal transcribed spacer ITS) region (ITS1, 5.8S, and ITS2) was preferred over the small subunit (SSU) rDNA as it was shown to be more informative and was comparable to both European (Puechmaille et al., 2010; Wibbelt et al., 2010) and North American *G. destructans* (Blehert et al., 2009, Chaturvedi et al., 2010). All sequences obtained were identical and showed 100% similarity with previously published ITS sequences of *G. destructans* available on GenBank (retrieved on

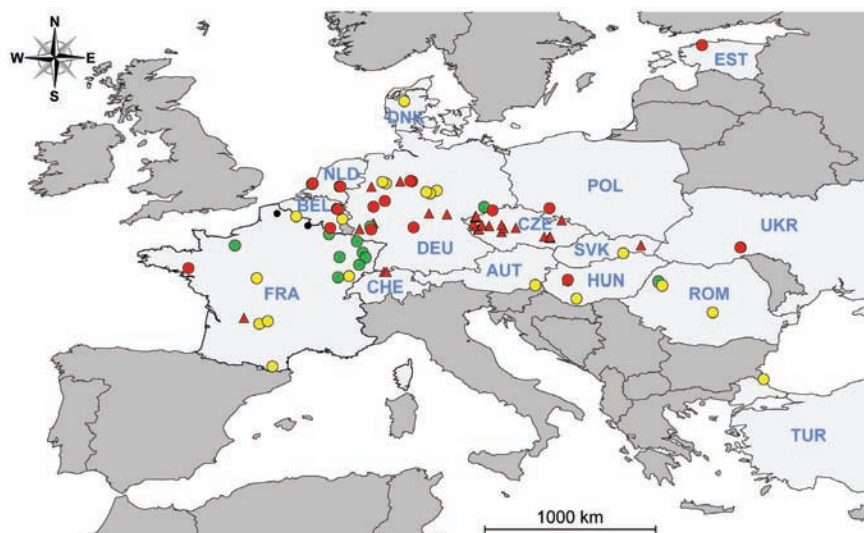


FIGURE A21-1 Distribution of confirmed and suspected records of *G. destructans* on hibernating bats in Europe. Data are presented for genetically confirmed records of *G. destructans* in red (circles, this study; triangles, published records), photographic evidence in yellow, visual reports in green. Dead bats from Northern France which culture and genetic analysis did not reveal the presence of *G. destructans* are depicted as black dots. Countries abbreviated names are as follows: AUT: Austria, BEL: Belgium, CHE: Switzerland, CZE: Czech Republic, DEU: Germany, DNK: Denmark, EST: Estonia, FRA: France, HUN: Hungary, NLD: Netherlands, POL: Poland, ROM: Romania, SVK: Slovakia, TUR: Turkey, UKR: Ukraine. doi:10.1371/journal.pone.0019167.g001.

October 13th) (Puechmaille et al., 2010; Blehert et al., 2009, Wibbelt et al., 2010; Chaturvedi et al., 2010).

Seasonal distribution of G. destructans

The monitoring of one site over two consecutive winters showed an absence of *Gd*-suspect bats from September until the end of January (Figure A21-S1). The first *Gd*-suspect bats were reported in February each year (16/02/2007 and 07/02/2008) and their numbers peaked in March (Figure A21-3). In April, the total number of bats and the number of *Gd*-suspect bats decreased as bats left the hibernacula. However, as the number of *Gd*-suspect bats decreased more slowly than the total numbers of bats, the highest prevalence was observed in April (Figure A21-S1). Prevalence varied between years for the same period of the year and reached values in the range of 18–25% in 2007 (14th–28th March)



FIGURE A21-2 (facing page). Photographic evidence showing bats with confirmed or suspected growth of *G. destructans*. Photographs of cases confirmed by genetic analysis, from (A) Estonia (*M. brandtii*, May 23rd 2010, © L. Lutsar), (B) Poland (*M. myotis*, March 7th 2010, © A. Wojtaszewski), (C) Germany (*M. myotis*, March 10th 2010, © C. Jungmann), (D) France (*M. myotis*, March 4th 2010, © Y. Le Bris), (E) Netherlands (*M. daubentonii*, March 9th 2010, © T. Bosch), (F) Germany (*M. myotis*, March 23rd 2010, © K. Passior) (G) Belgium (*M. mystacinus*, March 18th 2010, © B. Mulken), (H) Germany (*M. mystacinus*, March 23rd 2010, © K. Passior) or bats with white-fungal growth suspected as *G. destructans* from (I) Denmark (*M. dasycneme*, March 14th 2010, © B. Ohlendorf), (J) Austria (*M. myotis*, February 2nd 2007, © O. Gebhardt), (K) Hungary (*M. myotis*, February 19th 2010, © T. Görföl), (L) Belgium (*M. myotis*, March 7th 2010, © F. Forget), (M) France (*M. myotis*, February 13th 2010, © J. Vittier), (N) Ukraine (*M. myotis*, February 13th 2010, © A.-T. Bashta), (O) France (*M. escaleraei*/sp. A, June 25th 2010, © F. Blanc), (P) Turkey (*M. myotis/blythii*, March 22nd 2009, © M. Doker), and (Q) Romania (*M. blythii*, March 29th 2008, © B. Szilárd).

doi:10.1371/journal.pone.0019167.g002

or 28–55% in 2008 (13th–28th March) when the numbers of *Gd*-suspect bats are at the highest. The distributions of reported cases were similar between the two years, although more cases were reported in April in the winter 2007/2008 (Figure A21-3). In April 2008, the monitoring of three marked bats with white fungal growth clearly showed that after a bat had changed its position within the hibernaculum or when it was leaving the hibernaculum, the visible white fungal growth disappeared (Figure A21-4), most likely as a result of self-grooming.

The temporal distribution of reported cases of live *Gd*-suspect bats from throughout Europe (this study, $n = 105$) was combined with information available from previously reported cases of *G. destructans* (Puechmaille et al., 2009; Wibbelt et al., 2010) ($n = 22$) to investigate the seasonal variation across multiple sites in Europe. The temporal range of reported cases of *Gd*-suspect live bats and bats confirmed with *G. destructans* ($n = 127$) was not evenly distributed throughout the winter/ spring, with about 2/3rd of the cases reported in March (81/127; Figure A21-3). The number of reported cases more than doubled between February (30 cases) and March (81 cases). The earliest case was reported on January 17th from Belgium and the three latest cases were observed on May 23rd in Estonia, in June 24th in the Netherlands and June 25th in France (Tables A21-1, A21-2, A21-3, Figures A21-2A and A21-2).

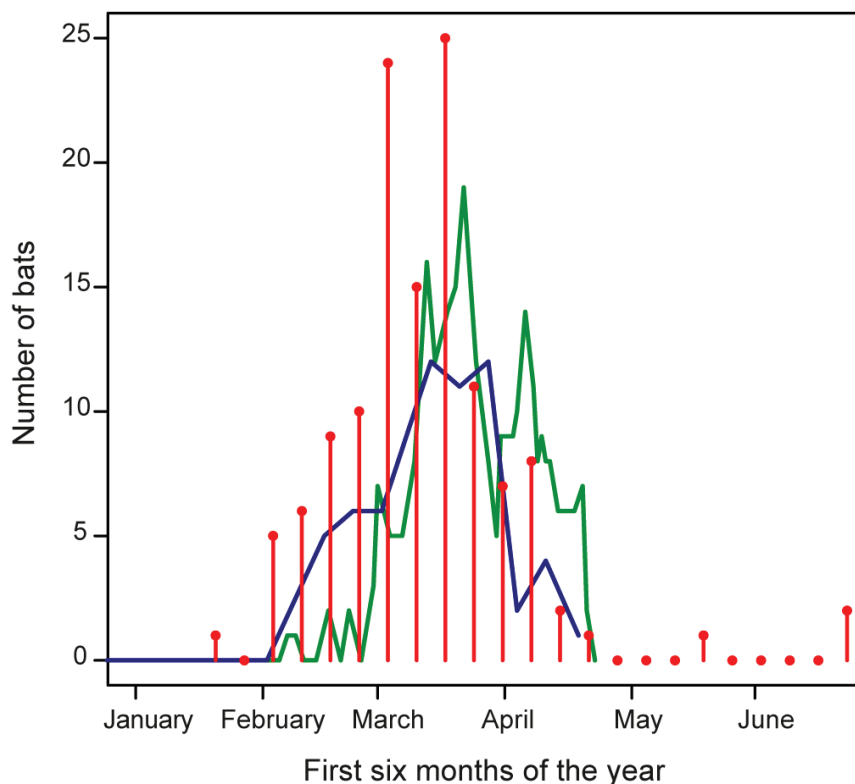


FIGURE A21-3 Seasonal changes of the number of live bats reported with white fungal growth in Europe. The number of bats with visible white fungal growth at an hibernaculum in Germany was monitored during the winter 2006/2007 (blue line) and the winter 2007/2008 (green line). The vertical red lines represent the number of *Gd*-suspect bats (or confirmed) observed across twelve European countries ($n = 127$) from 2003 until 2010. In the X-axis, the thick marks represent the start of each month. doi:10.1371/journal.pone.0019167.g003

Discussion

Presence of G. destructans in Europe

G. destructans was first identified in Europe in 2008–2009 (Puechmaille et al., 2009; Wibbelt et al., 2010) but increasing photographic evidence suggest that the fungus was present in Europe well before this date (this study, [Martínková et al., 2010; Feldmann 1984]). Most previous studies investigating fungi in European caves, including bat guano (Nováková, 2009; Mosca and Campanino, 1962; Groth et al., 1999; Nováková and Kolařík, 2010) reported *Geomyces* species, but none had curved conidia so far typical of *G. destructans*. In the Czech Republic,

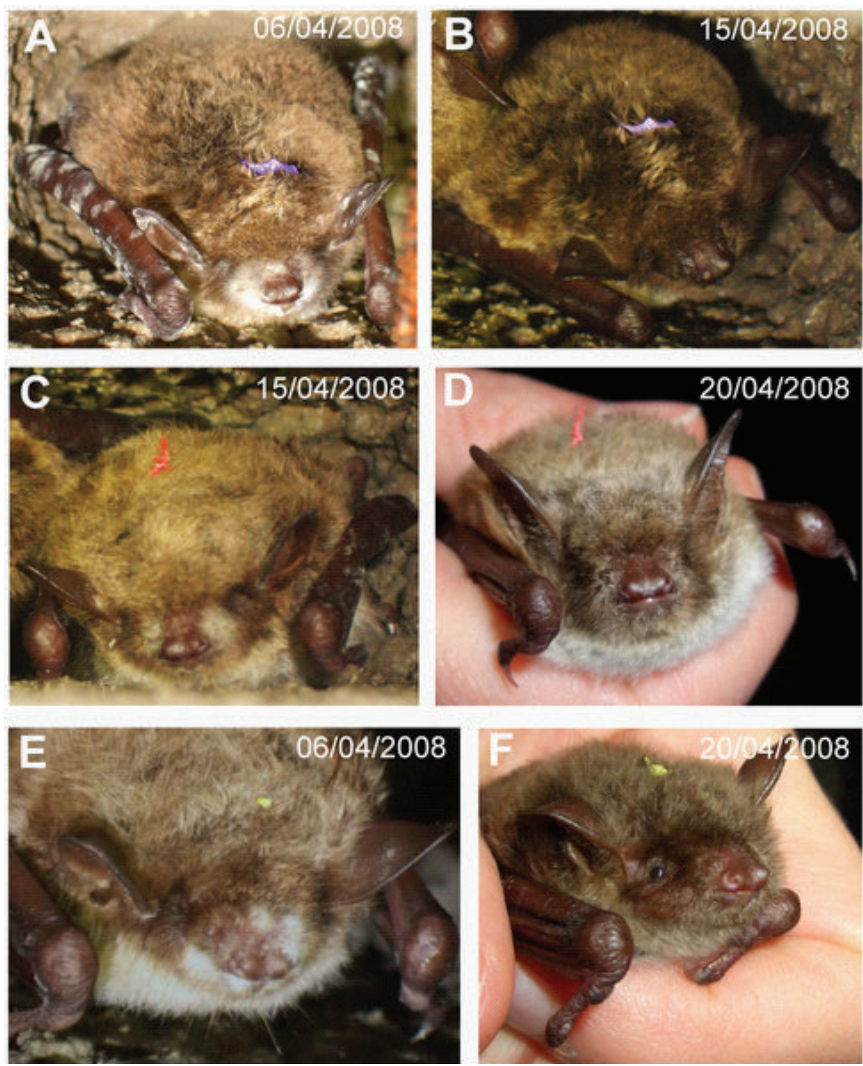


FIGURE A21-4 Indirect evidence of bats grooming off *G. destructans* during hibernation. Photographic evidence showing three different *M. dasycneme* individuals (A–B, C–D and E–F) observed at two different dates, first with visible fungal growth (A, C, E) and later without visible fungal growth (B, D, F). The bat in A–B changed its position within the hibernaculum whereas the other two (C–D and E–F) were captured when leaving the hibernaculum (© V. Korn). doi:10.1371/journal.pone.0019167.g004

Kubátová & Dvořák (2005) investigated fungi associated with insects hibernating in underground sites but did not find *Geomyces* species. To our knowledge, only one study in Europe has investigated fungi present in bats' skin and hair samples where, based on our current knowledge, *G. destructans* is most likely to be found. During the winter 1999/2001, Larcher et al. (2003) collected 25 samples of hair and skin swabs from six species, including three *Myotis* *Myotis*, but did not find any *Geomyces* species. It is important to note that most fungal cultures have been carried out at temperatures above 24–25°C, temperatures at which *G. destructans* does not grow (Gargas et al., 2009; Chaturvedi et al., 2010), which could explain why although present, this fungal species had never been reported in Europe before the study of Puechmaille et al. (2010).

Combining previously published data from France, Germany, Switzerland, Hungary, The Czech Republic and Slovakia (Puechmaille et al., 2010; Martínková et al., 2010; Wibbelt et al., 2010), additional data collected from France, Germany and Hungary (this study), and new data from Belgium, The Netherlands, Poland, Estonia and Ukraine (this study), we demonstrate here that *G. destructans* is widespread in Europe. We consider the photographic evidence of bats with white fungus matching the characteristic growth pattern (e.g., Figure A21-2; pictures from Romania and Turkey) to most likely represent *G. destructans*, because so far all tested live European bats with such white fungal growth on their nose, similar to Figure A21-2, have been confirmed to carry that species of fungus. These findings further support the fact that *G. destructans* is widespread across Europe. However, to confirm the presence of *G. destructans* in Europe prior to 2008, historical collections of bat specimens (or cave soil samples), especially specimens collected during the hibernation period, should be screened for the fungus.

As depicted in Figure A21-1, most cases of bats with *G. destructans* (confirmed and suspected) have been found from North-eastern France through Belgium, The Netherlands, Germany and the Czech Republic. However, it is not clear whether this pattern reflects an actual higher occurrence and/or prevalence of the fungus in these regions or if it is at least partly due to sampling bias, whereby the fungus is more likely to be detected in regions with a higher number of underground sites visited every winter or in regions where the fungus is specifically sought. In our opinion, it is most likely that this large-scale pattern is due to a sampling bias. For example, the largest number of sites with *G. destructans* in any European country was reported from the Czech Republic (76 localities with suspected or confirmed *G. destructans*) where most sites have been searched for signs of the fungus (>800 hibernacula) (Martínková et al., 2010).

G. destructans growth on bats

The clear seasonal peak in the number of observations of bats with white fungal growth indicates an increasing prevalence or detectability of *G. destructans* as winter passes. This suggests that bats might acquire *G. destructans* late

during the hibernation period or that the fungus is carried by bats at the onset of hibernation but needs time to develop the visible white fungal growth due to the phenology of the fungus. Therefore, the absence of visible white fungal growth on bats when observed with the naked eye may not directly reflect the absence of *G. destructans*, but rather just the absence of visible fungal colonies. Further complicating matters, our ability to detect *G. destructans* growth on bats can substantially differ with proximity to the bats (i.e., low ceiling versus high ceiling) or the location of the bat (ceiling versus crevices).

Our results confirm the suggestion of Martínková et al. (2010) by showing that during the hibernation period, bats can remove the fungus from their snout, ears and wings to a point where the fungus is no longer visible to the naked eye, although some spores might still be present on their skin. During hibernation, bats arouse every two weeks on average (Brack and Twente, 1985; Twente et al., 1985) and if bats consistently groom off the fungus on these occasions, our ability to visually detect the fungus, if present, will be considerably reduced. We also showed that towards the end of the hibernation period, bats were emerging from the hibernaculum without visible signs of the fungus despite showing visible white fungal growth from two weeks to five days before leaving the hibernaculum. It would be important to investigate whether bats carry spores out of hibernacula and as a result could possibly contaminate maternity roosts and maternity mates as suggested by Hallam and McCracken (2011).

Factors affecting G. destructans prevalence

Although it is not possible to clearly identify the mechanism responsible for the sudden increase in the prevalence of *G. destructans* in late February and March, these data suggest that shorter winter periods should be associated with lower prevalence. This prediction seems to hold as in the Mediterranean region, where hibernation periods are shorter (Rodrigues, 2003), no bats with visually conspicuous fungal growth have yet been reported during winter cave surveys. The case reported from Southern France (June 25th 2010, Figure A21-2) was found in the Pyrenees mountains at ca. 1700 m a.s.l. and hence, is not considered typical of the Mediterranean climate. It is nevertheless too early to conclude on this association between *G. destructans* prevalence and the hibernation duration, as other factors would need to be considered such as for example, the higher temperature observed in hibernacula in the Mediterranean region compared to other regions in Europe (Rodrigues, 2003). Higher temperatures in hibernacula have been associated with more frequent arousals in *Rhinolophus ferrumequinum* (Ransome, 1971; Arlettaz et al., 2000; Park et al., 2000). Considering that this association holds for other species, as a consequence of more frequent arousals, bats are expected to groom more often and therefore, reduce the probability of a visible fungal growth to develop. More surveys and strategic sampling efforts are needed to uncover whether the length of the hibernation period and/or climatic

conditions have a direct or indirect effect on the growth rates, prevalence, and detectability of *G. destructans* on bats.

It is crucial that the change in prevalence or detectability over the hibernation period is considered when comparing prevalence across sites and/or years. Our results from monitoring one site throughout the hibernation period over two consecutive years as well as reported cases from multiple sites in multiple years show that bats with fungal growth are first seen in January, the number of cases slowly increases into February and peaks in March, then in April when bats emerge from hibernation it drops again. Our results are in agreement with recent results from the Czech Republic where in the winter 2009/2010, the number of sites with bats with white fungal growth increased from 4.1% in January/February (33/800 sites; regular bat monitoring) to 77.5% in late February/March (76/98 sites; additional inspections) (Martínková et al., 2010). The Czech study reported that this increase in *G. destructans* prevalence was “*suggestive of an epizootic spread of the fungus*” (Martínková et al., 2010); we propose an alternative explanation whereby the increase in prevalence of *G. destructans* in late winter (March) might regularly (yearly) occur in Europe but has gone unnoticed. Nearly all hibernation counts in previous years were carried out between December and mid-February when prevalence/detectability of *G. destructans* is low, but not in March (Battersby, 2010) when the prevalence/detectability of *G. destructans* is at its highest (Figure A21-3). Although the total numbers of bats in the hibernacula decreased through April as bats left for the maternity colonies, our results show that there is a high probability of fungal growth developing on the remaining individuals. This further supports our hypothesis proposed above and links the duration of the hibernation period with the prevalence of *G. destructans*. By increasing the sample size, some cases might be reported earlier in the hibernation season or later through the summer, but we expect that the general pattern observed will not change. Despite these difficulties in assessing the occurrence of the fungus on bats, our data are consistent with other studies (Puechmaille et al., 2010; Martínková et al., 2010; Wibbelt et al., 2010), and also demonstrate that the most commonly encountered bat species with *G. destructans* in Europe is the largest species of *Myotis* on the continent, *Myotis myotis*. In countries/regions (i.e., the Netherlands, Northwest Germany) where *M. dasycneme* is more commonly encountered in hibernacula, *G. destructans* prevalence can reach high levels in that species. It is interesting to note that neither *Pipistrellus pipistrellus* nor *Miniopterus schreibersii* have been observed with *G. destructans* (Puechmaille et al., 2010; Martínková et al., 2010; Wibbelt et al., 2010), although these two species are known to hibernate in aggregations of tens of thousands of individuals, especially the latter (Furman and Özgül, 2004; Nagy and Postwana, 2011; Benda et al., 2003; Serra-Cobo et al., 1998). Although rare, hibernacula of a few thousands and up to about 34,000 individuals are also known for species of *Myotis* in Europe (Furman and Özgül, 2004; Nagy and Postwana, 2011; Kokurewicz, 2009; Arthur and Lemaire, 2009; Sachanowicz et al., 2006; Dietz et al., 2009).

G. destructans outside of the hibernation period

We observed three individual bats with white fungal growth around their nose (one confirmed as *G. destructans*) from May and June, when they were still torpid in cold underground sites. This represents the first mention of individuals with *G. destructans* colonisation outside of the hibernation period and raises questions about the role of these individuals in the persistence of the fungus in bat populations. *Pipistrellus pipistrellus* During the summer period, while females aggregate in colonies to raise their young, it remains largely unknown where males are roosting (e.g., Senior et al., 2005). Furthermore, during the swarming season in late summer/autumn, large numbers of individuals aggregate in caves, mines or tunnels and come in close contact with each other (chasing, mating) (Senior et al., 2005; Parsons and Jones, 2003; Parsons et al., 2003a; Parsons et al., 2003b; Rivers et al., 2006; Rivers et al., 2005), which could represent an opportunity for *G. destructans* to be transmitted between individuals.

We isolated *G. destructans* from the environment surrounding hibernating bats. The presence of viable spores of *G. destructans* on the surfaces of hibernation sites has huge implications for the understanding of disease transmission mechanisms and disease modelling (Hallam and McCracken, 2011). It seems likely that cave walls could serve as a passive vector and/or reservoir for *G. destructans* spores. It is not yet known how long these spores can remain viable but fungal spores generally remain viable for extended periods. Bats entering these sites in autumn (for swarming and/or hibernation) could become contaminated with spores of *G. destructans* left from bats infected during the previous winter. In North-America, Lindner et al. (2010) successfully amplified ITS sequences identical to *G. destructans* DNA from soil samples collected during the winter 2008–2009 at three bat hibernacula and stressed the importance of considering the environment as a reservoir for *G. destructans* and in the dynamics of WNS transmission. Our results confirm this and further suggest that more work is needed to understand the persistence of *G. destructans* on hibernacula walls (reservoir or passive vector) where they are in physical contact with bats.

Insights into the origin of *G. destructans* and WNS

The wide distribution of *G. destructans* in Europe and the absence of associated mortality supports the hypothesis that *G. destructans* has co-evolved with European bats and only recently arrived in North America where it is causing unprecedented mass mortalities (Puechmaille et al., 2010; Blehert et al., 2009; Martínková et al., 2010; Wibbelt et al., 2010). Alternatively, *G. destructans* could have been present on both continents and a virulent strain could have evolved in North-America. Until the relationships between *G. destructans* populations across continents are clarified, precautions should be taken to minimise the chances of transcontinental movement of viable *G. destructans* (Puechmaille et al., 2011).

During the two years monitoring at one site in Germany where *G. destructans* prevalence reached high levels in March-April, not a single dead bat was found. This is in agreement with previous studies (Puechmaille et al., 2010; Martínková et al., 2010; Wibbelt et al., 2010) reporting that the presence of *G. destructans* in bats from Europe is not associated with mass mortality. This sharply contrasts with mass mortalities reported in North America where hundreds or thousands of dead bats are found in hibernacula towards the end of the hibernation period. Recent pathological investigations of bats dying from WNS in North America led Cryan et al. (2010) to propose that mortality was caused by important disruptions of wing-dependant physiological functions due to infection by *G. destructans*. In North America, the fungus deeply invades wings tissues (Meteyer et al., 2009) and causes damages that are thought to alter homeostasis and water balance, resulting in more frequent arousals than bats can afford with their fat reserves, leading to death by starvation (Cryan et al., 2010). The pathology associated with *G. destructans* colonisation in Europe is not yet known. We believe that the first step in understanding mortality differences between bats from Europe and North America rely on understanding pathological differences incurred by the fungus on the bats' wings. As a result, we urge the necessity to carry out pathological investigation of live bats from Europe colonised by *G. destructans*. Despite the absence of mortality associated with the presence of *G. destructans* in Europe, it would be necessary to investigate whether chronic infections with the fungus are compromising the health of individuals, especially in *M. Myotis* and *M. dasycneme*, which show high prevalence of the fungus towards the end of the hibernation period.

Phylogeographic studies of European bat species have shown that in the last 100,000 years, some species colonised Europe from Western Asia (Flanders et al., 2009), including *Myotis blythii* (Berthier et al., 2006; Currat et al., 2008) which has been found with *G. destructans* (Wibbelt et al., 2010). Assuming that *G. destructans* can be transported over long distances by bats, we speculate that the distribution of *G. destructans* is probably not limited to Europe and possibly extends eastwards into Russia, Western and Central Asia. Further surveys are necessary to clarify the global distribution of *G. destructans*.

Conclusions

We have shown here that *G. destructans*, the most likely causative agent of WNS in North America, is widespread in Europe, but is not associated with mass mortality. The prevalence of visible fungal growth on bats increases in February/March before sharply decreasing when bats emerge from hibernation. We also isolated viable *G. destructans* from the walls of an underground site suggesting that the hibernacula could act as passive vectors and/ or reservoirs for *G. destructans* and therefore, might play an important role in the transmission process. Further research is needed to clarify the global prevalence of *G. destructans* and

identify variables (e.g., temperature, humidity and hibernation length) explaining regional differences. Finally, further research is needed in different parts of the globe, especially temperate region of the Northern and Southern hemispheres, to precisely determine the global distribution of *G. destructans*.

Materials and Methods

Sample collection

During ongoing population censuses carried out at hibernacula in different countries across Europe and during additional hibernacula surveys carried out for the purpose of this study, information on bats with visible white fungal growth on snouts and/or ears was recorded. Whenever possible, sterile dry cotton swabs (Puechmaille et al., 2010) or adhesive tape touch imprints (Wibbelt et al., 2010) were used to collect fungal material from the bats. In Estonia, samples were collected from the wall of the tunnel where a bat with characteristic white fungus was observed nine days prior to the sampling. Where no sample collection was possible, a photograph was taken of the bat (photographic record). In cases where neither sample collection nor photographic evidence was obtained, the record was classified as visual observation. Live hibernating bats with powdery, white fungal growth on their noses were considered suspects of infection by *G. destructans* (*Gd*-suspects) but not suspected of having WNS. There is presently no data supporting the occurrence of WNS in Europe and the co-occurrence of the fungus with lesions characteristic of WNS (Meteyer et al., 2009) has not (yet) been reported in Europe (Wibbelt et al., 2010; Barlow et al., 2005). Although, prevalence of *G. destructans* can reach high levels in some European species (i.e., *Myotis myotis*, *M. dasycneme*) in late winter (especially in March), it can be expected that by chance alone some bats dying from causes unrelated to the presence of *G. destructans* will also be carrying the fungus. Unless the criteria for the diagnosis of WNS are met (confirmation by histo-pathology and PCR) (Meteyer et al., 2009) WNS should not be assumed as a cause of mortality in dead bats found in hibernacula of Europe. Various species of fungi have been identified on dead bats (Wibbelt et al., 2010; Voyron et al., 2011), most of them likely being saprophytes that colonise bat carcasses *post-mortem*.

Fungal cultures

In the laboratory, samples were treated as in Puechemaille et al. (2010) for swabs and following Wibbelt et al. (2010) for touch imprints. Briefly, swabs were streak-plated onto plates of Sabouraud's agar, supplemented with 0.1% mycological peptone. For touch imprints, small areas with fungal conidia characteristic of *G. destructans* were identified by light microscopy and the tape was disinfected and excised before being transferred for culture to Sabouraud's agar.

The plates were sealed with parafilm and incubated inverted in the dark at 10°C. A fungal growth developed within 14 days, from which single spore cultures were established.

Molecular identification

Each culture was sequenced for one molecular marker, the rRNA gene internal transcribed spacer (ITS, *ca.* 930 bp.) region (ITS1, 5.8S, and ITS2) to further confirm species identity. The DNA extraction, PCR amplification and DNA sequencing followed protocols described in Puechmaille et al. (2010). Briefly, DNA was extracted using the Qiagen Blood and Tissue kit following the manufacturer's instructions with slight modifications (after step 3, we added an incubation time of 10 minutes at 70°C). PCR reactions were carried out in 25 mL containing 1 mL of DNA extract (at 10–75 ng/mL), 1.5 mmol/L MgCl₂, 0.4 mmol/L each primer (Forward: ITS4, 5'-TCCTCCGCTTATTGATATGC – 3'; Reverse: ITS5, 5'-GGAAGTAAAAGTCGTAACAAGG – 3'; (White et al., 1990), 0.2 mmol/L dNTP, 1x PCR buffer and 1 U Platinum Taq DNA Polymerase High Fidelity (Invitrogen). PCR cycling conditions were: initial step 15' at 95°C, then 10 cycles of 30" at 95°C, 1'45" at 60°C (reduce of 2°C every 2 cycles), 1' at 72°C, following by 30 cycles of 30" at 95°C, 1'45" at 50°C and 1' at 72°C. PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea) in both directions using the PCR primers. Complementary sequences were assembled and edited for accuracy using CodonCode Aligner 3.0.3 (www.codoncode.com/aligner/download.htm).

Monitoring of visible fungal growth on bats

One site situated in Northwest Germany (Latitude: 52.1; Longitude: 8.2) near the city of Osnabrück was monitored over two consecutive winters, 2006/2007 (5th September until 19th May) and 2007/2008 (28th August until 23rd April). The monitoring consisted of counting the total number of bats at the site as well as the number of bats with visible white fungal growth similar to the pictures presented in Figures A21-2 and A21-4. The counts were done by the same person (V. Korn) every 4 days on average during the first year and every 2.5 days on average during the second year. The procedures complied with guidelines of the American Society of Mammalogists and were carried out under permit number FBD7.2 60 from the Administration of the County of Osnabrück, Department of Environment.

Supporting Information

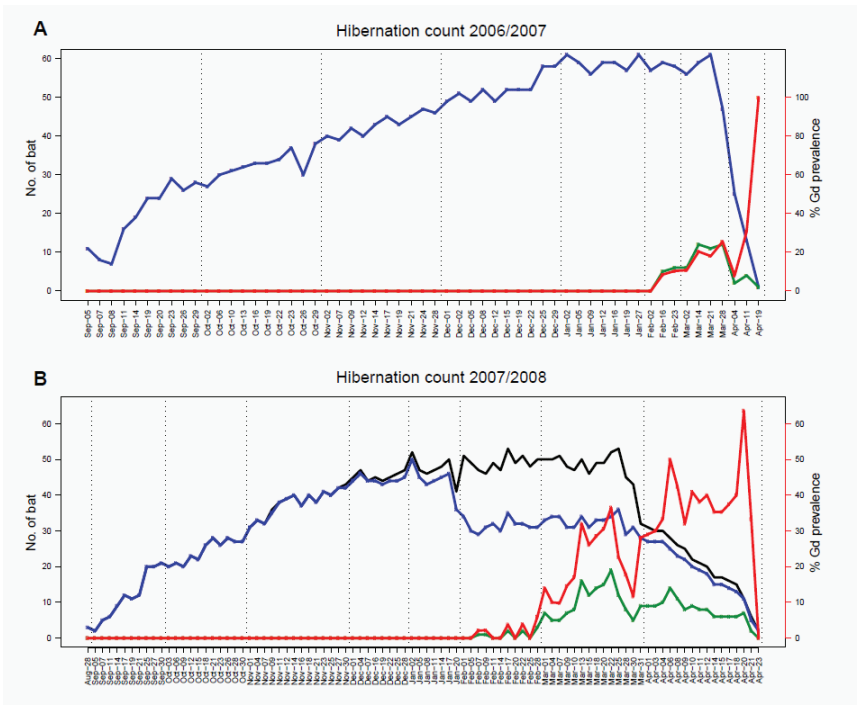


FIGURE A21-S1 Monitoring of bats at an hibernaculum in Germany during (A) the winter 2006/2007 (September 5th 2006 until April 19th 2007) and (B), the winter 2007/2008 (August 28th 2007 until April 23rd 2008). The blue line represents the total number of bats counted whereas the green line represents the number of bats with visible white fungal growth (*Gd*-suspects). Dotted vertical lines separate counts from each month. Note that the number of counts per month was not equal between months. In (B), the black line represents the total number of bats counted whereas the blue line represents the total number of bats bar one portion of the hibernaculum where bats grouped densely (ca. 20 individuals) and did not allow a reliable identification of the number of bats with white fungal growth. The green line represents the number of bats with visible white fungal growth (*Gd*-suspects) counted at the hibernaculum without considering individuals densely grouping at one place in the hibernaculum. The group of about 20 individuals formed while the hibernaculum was partially flooded, likely as a result of bats changing position to avoid drowning. Note that the right Y-axis scale is different between (A) and (B).

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Author Contributions

Conceived and designed the experiments: SJP GW VK ECT. Performed the experiments: SJP GW HF VK KM AK. Analyzed the data: SJP GW. Contributed reagents/materials/analysis tools: SJP GW VK HF KM AK FF WB CB TB TC MD TG AJH FH GH MH CJ YLB LL MM BM KP MS AW UZ ECT. Wrote the paper: SJP GW.

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Appendix B

Agenda

Fungal Diseases: An Emerging Challenge to Human, Animal, and Plant Health

December 14–15, 2010
Keck Building, Room 100
500 Fifth Street, NW
Washington, DC

DAY 1: DECEMBER 14, 2010

- 8:00–8:30: Registration and Continental Breakfast
- 8:30–8:45: Welcoming Remarks
David A. Relman, M.D., Chair, and James M. Hughes, M.D.,
Vice Chair, Forum on Microbial Threats
- 8:45–10:15: Keynote Remarks
James M. Hughes, Moderator
- 8:45–9:30: The “Good,” the “Bad,” and the “Ugly”: Fungi Mold Your
World
Meredith Blackwell, Ph.D., Louisiana State University

9:30–10:15: Emerging Fungal Pathogens—Past, Present, and Future
Arturo Casadevall, M.D., Ph.D., Albert Einstein College of
Medicine

10:15–10:45: Discussion

10:45–11:00: Break

Session I
Environmental Factors Influencing the Emergence
and Spread of Fungal Diseases

Moderator: Steven Brickner, Ph.D.

11:00–11:30: Climate, Extreme Weather Events, and Fungal Disease
Emergence and Spread
Compton (Jim) Tucker, Ph.D., NASA Goddard Space Flight
Center

11:30–12:00: Trade-Mediated Spread of Fungal Disease and Resulting Shifts
in Amphibian Conservation Status and Practices
Ché Weldon, Ph.D., North-West University

12:00–12:30: Weather, Globalization, and Trade—Impacts on Invasion and
Dispersal of Fungal Plant Pathogens
Jim Stack, Ph.D., Kansas State University
(for Michael Jeger, Ph.D., Imperial College London)

12:30–1:00: Geography, Climate, Dust, and Disease—Epidemiology of
Valley Fever (*Coccidioides Spp.*) and Ways It Might Be
Controlled
John Galgiani, M.D., University of Arizona

1:00–1:20: Discussion

1:20–2:00: Lunch

Session II
Case Studies of Emerging Fungal Diseases
of Humans, Animals, and Plants

Moderator: Erica Rosenblum, Ph.D.

- 2:00–2:30: Predicting and Mitigating Mycoses by Merging Deep Sequencing with Global Mapping Projects (Amphibian Chytridiomycosis)
Matthew Fisher, Ph.D., Imperial College London
- 2:30–3:00: Bat White-Nose Syndrome: An Emerging Fungal Pathogen in New World Bats
David Blehert, Ph.D., National Wildlife Health Center, U.S. Geological Survey
- 3:00–3:30: Knowing Where to Look—Environmental Sources of Cryptococcal Disease in Human and Animal Residents in the Pacific Northwest
Karen Bartlett, Ph.D., University of British Columbia
- 3:30–3:45: Break
- 3:45–4:15: Emergence of *Phytophthora ramorum* in Europe and North America
David Rizzo, Ph.D., University of California–Davis
- 4:15–4:45: Rapid Global Spread of Aggressive Strains of *Puccinia striiformis* on Wheat—Origins, Causes, and Consequences
Mogens Støvring Hovmøller, Ph.D., Aarhus University
- 4:45–5:15: Discussion
- 5:15–6:00: Open Discussion of Day One
- 6:00: Meeting Adjourns

DAY 2: DECEMBER 15, 2010

- 8:30–9:00: Continental Breakfast
- 9:00–9:15: Summary of Day One: David A. Relman, M.D., Chair, Forum on Microbial Threats

Session III**Host and Pathogen Factors Influencing the Emergence and Spread of Fungal Diseases**

Moderator: Jeff Duchin, M.D.

- 9:15–9:45: Fungal Pathogenesis in Plants and Animals: Similarities and Differences
Barbara Howlett, Ph.D., University of Melbourne
- 9:45–10:15: Sexual Reproduction, Evolution, and Adaptation of *C. gattii* in the Pacific Northwest
Joseph Heitman, M.D., Ph.D., Duke University
- 10:15–10:30: Break
- 10:30–11:00: Genetic Factors and Immune Responses to Fungal Infection in Health and Disease
Steve Holland, M.D., National Institute of Allergy and Infectious Diseases
- 11:00–11:30: Host–Pathogen Dynamics of Amphibian Chytridiomycosis: The Role of the Skin Microbiome in Health and Disease
Vance Vredenburg, Ph.D., San Francisco State University
- 11:30–12:00: *Geomyces destructans* in Old World and New World Bats
Gudrun Wibbelt, D.V.M., Leibniz Institute for Zoo and Wildlife Research
- 12:00–12:30: Discussion
- 12:30–1:15: Lunch

Session IV
Surveillance, Detection, and Response

Moderator: Steve Morse, Ph.D.

- 1:15–1:45: Informal Surveillance Networks for Diseases of Humans, Plants, and Animals
Larry Madoff, M.D., Massachusetts Department of Public Health & University of Massachusetts Medical School
- 1:45–2:15: Local and National Detection Capability and Public Health Responses for *C. gattii*
Julie Harris, Ph.D., Centers for Disease Control and Prevention
- 2:15–2:45: Global Capacity for Coordinated Surveillance, Detection, and Response to Emerging Diseases of Wildlife
Peter Daszak, Ph.D., EcoHealth Alliance
- 2:45–3:15: Wildlife Conservation at the Smithsonian Conservation Biology Institute
Luis Padilla, D.V.M., Smithsonian Conservation Biology Institute
- 3:15–3:45: Break
- 3:45–5:15: Panel and Open Discussion
Speakers: Peter Daszak, Julie Harris, Larry Madoff, and Luis Padilla
Discussants: David Blehert, Jacque Fletcher, and Kevin Russell
- 5:15–5:30: Closing Remarks
James M. Hughes and David A. Relman
- 5:30: Meeting Adjourns

Appendix C

Acronyms

AARK	Amphibian Ark
ACAP	Amphibian Conservation Action Plan
AFTOL	Assembling the Fungal Tree of Life program
AM	Arbuscular mycorrhizae
APHIS	Animal and Plant Health Inspection Service
B.C.	British Columbia
BCCDC	British Columbian Centre for Disease Control
<i>Bd</i>	<i>Batrachochytrium dendrobatidis</i>
BGRI	Borlaug Global Rust Initiative
BWNS	bat white-nose syndrome
CANV	<i>Chrysosporium</i> anamorph of <i>Nanniziopsis vriesii</i>
CBD	Convention on Biological Diversity
CDC	Centers for Disease Control and Prevention
CFU	colony-forming unit
CGB	canavanine-glycine-bromothymol blue
CGD	chronic granulomatous disease
CGIAR	Consultative Group for International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Center
CITIES	Convention for International Trade of Endangered Species
DNA	deoxyribonucleic acid
DoD	Department of Defense

DoD-GEIS	Department of Defense-Global Emerging Infections Surveillance and Response System
DOI	Department of the Interior
DTRA	Defense Threat Reduction Agency
EID	emerging infectious disease
ELISA	enzyme-linked immunosorbent assay
ENSO	El Niño-Southern Oscillation
EUS	epizootic ulcerative syndrome
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FWS	Fish and Wildlife Service
GAA	Global Amphibian Assessment
GAO	Government Accountability Office
GHI	Global Health Initiative
GOARN	Global Outbreak and Response Network
GPHIN	Global Public Health Intelligence Network
GPM	Gaussian plume model
GRACE	Gravity Recovery and Climate Experiment
GRC-YR	Global Reference Center for yellow rust
GRRC	Global Rust Reference Center
HIV/AIDS	human immunodeficiency virus/acquired immune deficiency syndrome
ICARDA	International Center for Agricultural Research in the Dry Areas
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IHR	International Health Regulations
IOM	Institute of Medicine
IRIS	immune reconstitution inflammatory syndrome
IRRI	International Rice Research Institute
ITS	internal transcribed spacer
IUCN	International Union for the Conservation of Nature
MIC	minimum inhibitory concentration
MLST	multilocus sequencing typing
NAS	National Academy of Sciences
NASA	National Aeronautics and Space Administration
NGO	non-governmental organization
NIAID	National Institute of Allergy and Infectious Diseases

NIH	National Institutes of Health
NLM	National Library of Medicine
NOAA	National Oceanographic and Atmospheric Administration
NSF	National Science Foundation
NWHC	National Wildlife Health Center
OIE	World Organisation for Animal Health
OSTP	Office of Science and Technology Policy
PAMP	pathogen-associated molecular pattern
PCR	polymerase chain reaction
PNW	Pacific Northwest
ProMED	Program for Monitoring Emerging Diseases
RACE	Risk Assessment of Chytridiomycosis to European Amphibian Diversity
RVF	Rift Valley fever
SCBI	Smithsonian Conservation Biology Institute
SCLB	Southern corn leaf blight
SOD	sudden oak death
SSC	Species Survival Commission
TB	tuberculosis
TGen	Translational Genomics Research Institute
U.K.	United Kingdom
U.N.	United Nations
USAID	U.S. Agency for International Development
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey
WHO	World Health Organization
WNS	white-nose syndrome
WTO	World Trade Organization

Appendix D

Glossary

Abiotic: Nonliving chemical and physical factors in an environment.

Adaptive immune response: Response of the vertebrate immune system to a specific antigen that typically generates immunological memory.

Aggressive: In plant pathology, the quality of being able to cause more disease more quickly on susceptible host plants.

Anthropogenic: Caused or produced by humans.

Antibiotic: Class of substances that can kill or inhibit the growth of some groups of microorganisms. Used in this report to refer to chemicals active against bacteria. Originally antibiotics were derived from natural sources (e.g., penicillin from molds), but many currently used antibiotics are semi-synthetic and modified with additions of manmade chemical components.

Antibody therapy: Any therapeutic intervention in which a monoclonal or other concentrated antibody is used to manage a condition, such as cancer or severe infection.

Antifungal: Substances that can kill or inhibit the growth of fungal organisms.

Ascomycete: Any of various fungi belonging to the phylum Ascomycota, characterized by the presence of sexually produced spores formed within an ascus. Like

most fungi, ascomycetes also reproduce asexually by the formation of nonsexual spores called conidia at the ends of filaments known as hyphae.

Assembling the Tree of Life program: This National Science Foundation program was created in 2004 with the goal of constructing the evolutionary history for all major lineages of life. The program supports efforts to classify all major groups of organisms and to reveal the pattern of historical relationships that would explain similarities and differences among them.

The program has three goals: (1) Creation and support of multidisciplinary teams of investigators to acquire and integrate molecular and morphological evidence on both extant and extinct organisms in order to resolve phylogenetic relationships of large, deep branches of the Tree of Life; (2) Research and development of tools for computational phylogenetics and phyloinformatics to improve assessment, predictive capabilities, and the visualization and navigation of the hierarchical structure in the Tree of Life; and (3) Outreach and education in comparative phylogenetic biology and paleontology.

Asymptomatic: Presenting no symptoms of disease.

Asymptomatic carriers: A person or animal that has contracted an infectious disease, but displays no symptoms yet has the ability to transmit it to others.

Bacteria: Microscopic, single-celled organisms that have some biochemical and structural features different from those of animal and plant cells.

Basidiomycetes: Any various fungi belonging to the phylum Basidiomycota, bearing sexually produced spores on a basidium. All hyphae of basidiomycetes are divided into segments by septa and go through three stages of development.

Biofuel: Fuel produced from renewable resources, especially plant biomass, vegetable oils, and treated municipal and industrial wastes.

Biological invasion: The process by which species (or genetically distinct populations), with no historical record in an area, breach biogeographic barriers and extend their range.

Bioluminescence: Light produced by a chemical reaction that originates in an organism.

Biota: The animal and plant life of a given region.

Chronic: Relating to an illness or a medical condition that is characterized by long duration or frequent recurrence.

Climate: Average meteorological conditions over a specified time period, usually at least a month, resulting from interactions among the atmosphere, oceans, and land surface. Climate variations occur over a wide range of spatial and temporal scales.

Climate change: A change of climate that is attributed directly or indirectly to human activity and alters the composition of the global atmosphere; this happens in addition to natural climate variability observed over comparable time.

Colonize: The spreading of a species into a new habitat.

Colony collapse disorder: A syndrome characterized by the disappearance of all adult honey bees in a hive while immature bees and honey remain.

Colony-forming unit: A standard unit of measurement for environmental sampling. Colonies reflect the number of “viable” organisms (i.e., organism capable of forming colonies when provided with nutritional elements necessary for growth).

Commensals: Organisms in a mutually symbiotic relationship where both live peacefully together while not being completely dependent on one another (e.g., the gut microbiome).

Communicable disease: An infectious disease transmissible (as from person to person) by direct contact with an affected individual or the individual’s discharges or by indirect means (as by a vector).

Conidia: Asexually produced fungal spore. Most conidia are dispersed by the wind and can endure extremes of cold, heat, and dryness. When conditions are favorable, they germinate and grow into hyphae.

Contagious: Capable of being transmitted by direct or indirect contact, as an infectious disease.

Corn Belt: The area in the Midwestern United States—roughly covering western Indiana, Illinois, Iowa, Missouri, eastern Nebraska, and eastern Kansas—in which corn (maize) and soybeans are the dominant crops.

Cultivar: A variety of a plant that has been created or selected intentionally and maintained through cultivation.

Detection: The act of discovering a novel, emerging, or reemerging disease or disease event.

Diagnosis: The identification of a condition, disease, or injury made by evaluating the symptoms and signs presented by an individual.

Dimorphic: The existence of two distinct types of individuals within a species, usually differing in one or more characteristics such as coloration, size, and shape.

Disease: A situation in which infection has elicited signs and symptoms in the infected individual; the infection has become clinically apparent. Some exposures to infectious disease-causing agents can also produce asymptomatic illnesses that can be spread to others.

Disease burden: The impact of a health problem in an area measured by financial cost, mortality, morbidity, or other indicators. It is often quantified in terms of quality-adjusted life years (QALYs) or disability-adjusted life years (DALYs), which combine the burden due to both death and morbidity into one index.

Ecosystem: A community of organisms together with their physical environment, viewed as a system of interacting and interdependent relationships and including such processes as the flow of energy through trophic levels and the cycling of chemical elements and compounds of the system.

Ecosystem services: Benefits derived from a multitude of resources and processes that are supplied by natural ecosystems (e.g., decomposition of waste, food, energy, nutrient dispersal, and cycling).

Emerging infectious disease: Infections that are rapidly increasing in incidence or geographic range.

Emigration: To leave one's usual country of residence to settle in another.

Endemic: Present in a community or common among a group of people; said of a disease prevailing continually in a region.

Endophytes: Fungi that live inside the plant tissue, but without causing any obvious negative effects.

Endosymbiont: An organism that lives inside another organism, most often for the benefit of the two (e.g., rhizobia [nitrogen-fixing soil bacterial] that live within root nodules—rhizobia cannot independently fix nitrogen, but need the plant as an energy source; in turn, rhizobia supply the plant host with ammonia and amino acids).

Environmental microbe: Microbe acquired from the environment (in contrast to acquisition from other living hosts).

Enzootic: A disease of low morbidity that is constantly present in an animal community.

Enzyme: Any of numerous proteins produced in living cells that accelerate or catalyze the metabolic processes of an organism.

Epidemic: The condition in which a disease spreads rapidly through a community in which that disease is normally not present or is present at a low level.

Epidemiology: Study of the distribution and determinants of health-related states or events in specified populations. Epidemiology is the basic quantitative science of public health.

Epizootic: A disease of high morbidity that is only occasionally present in an animal community.

Eradication: Reduction of the worldwide incidence of a disease to zero as a result of deliberate efforts.

Etiologic agent: The organism that causes a disease.

Etiological: Of or pertaining to causes or origins.

Etiology: Science and study of the causes of diseases and their mode of operation.

Eukaryotic organism: One of the three domains of life. The two other domains, Bacteria and Archaea, are prokaryotes and lack several features characteristic of eukaryotes (e.g., cells containing a nucleus surrounded by a membrane and whose DNA is bound together by proteins [histones] into chromosomes). Animals, plants, and fungi are all eukaryotic organisms.

Expression vectors: A plasmid that is used to introduce a specific gene into a target cell. Once the expression vector is inside the cell, the protein that is encoded by the gene is produced by the cellular-transcription and translation machinery ribosomal complexes. The plasmid is frequently engineered to contain regulatory sequences that act as enhancer and promoter regions and lead to efficient transcription of the gene carried on the expression vector.

Extreme weather: Refers to weather phenomena that are at the extremes of the historical distribution and are rare for a particular place and/or time, especially

severe or unseasonal weather. Such extremes include severe thunderstorms, severe snowstorms, ice storms, blizzards, flooding, hurricanes, high winds, and heat waves.

Fermentation: The process by which complex organic compounds, such as glucose, are broken down by the action of enzymes into simpler compounds without the use of oxygen.

Food security: The availability of food and one's access to it. A household is considered food secure when its occupants do not live in hunger or fear of starvation.

Fungi/fungal/fungus: For the purposes of this publication, the terms fungi, fungal, and fungus are used inclusively to describe all organisms traditionally studied by mycologists—including species that are now excluded from Kingdom Fungi (e.g., *Phytophthora ramorum* and *Phytophthora infestans*) or whose relationship to the fungal kingdom has yet to be determined (e.g., the microsporidia *Nosemus* spp.).

Genome: The complete genetic composition of an organism (e.g., human, bacterium, protozoan, helminth, fungus), contained in a chromosome or set of chromosomes or in a DNA or RNA molecule (e.g., a virus).

Genomics: The study of all the genes in a person, as well as interactions of those genes with each other and with that person's environment.

Genotype: The genetic makeup of an organism as distinguished from its physical characteristics.

Genus: A group of species with similar characteristics that are closely related.

Germinate: The beginning of growth, as of a seed, spore, or bud.

Globalization: The increased interconnectedness and interdependence of peoples and countries. It is generally understood to include two interrelated elements: (1) the opening of borders to increasingly fast flows of goods, services, finance, people, and ideas across international borders, and (2) the changes in institutional and policy regimes at the international and national levels that facilitate or promote such flows.

Haploid: Having a single set of each chromosome in a cell or cell nucleus. In most animals, only the gametes are haploid.

Hibernacula: A protective case, covering, or structure, such as a cave, in which an organism remains dormant for the winter.

Host: Animal or plant that harbors or nourishes another organism.

Hyphae: Slender tubes that develop from germinated spores and form the structural parts of the body of a fungus. A large mass of hyphae is known as a mycelium, which is the growing form of most fungi.

Immunocompetence: The ability of the immune system to respond appropriately to an antigenic stimulation.

Immunocompromised: A condition (caused, e.g., by the administration of immunosuppressive drugs or irradiation, malnutrition, aging, or a condition such as cancer or HIV disease) in which an individual's immune system is unable to respond adequately to a foreign substance.

Incidence: As used in epidemiology, the number of new cases of a disease that occur in a defined population within a specified time period; the rate of occurrence.

Incubation period: The time from the moment of inoculation (exposure to the infecting organism) to the appearance of clinical manifestations of a particular infectious disease.

Infection: The invasion of the body or a part of the body by a pathogenic agent, such as a microorganism or virus. Under favorable conditions the agent develops or multiplies; the results may produce injurious effects. Infection should not be confused with disease.

Innate immune response: Immune response (of both vertebrates and invertebrates) to a pathogen that involves the preexisting defenses of the body, such as barriers formed by skin and mucosa, antimicrobial molecules, and phagocytes. Such a response is not specific for the pathogen.

Inoculum: Collective term for microorganisms or their parts (spores, mycelial fragments, etc.), which are capable of infection or symbiosis when transferred to a host.

Internal transcribed spacer sequences: Internal transcribed spacer sequences are sections of non-functional RNA that are highly variable, even between closely related species, and are widely used for taxonomy purposes.

International Health Regulations (IHR): An international legal instrument that is binding on 194 countries across the globe, including all the Member States of the World Health Organization (WHO). Their aim is to help the international community prevent and respond to acute public health risks that have the potential to cross borders and threaten people worldwide.

The IHR, which entered into force on June 15, 2007, requires countries to report certain disease outbreaks and public health events to WHO. Building on the unique experience of WHO in global disease surveillance, alert, and response, the IHR define the rights and obligations of countries to report public health events, and establish a number of procedures that WHO must follow in its work to uphold global public health security.

Invasive species: Non-native plants and animals that, when introduced to new environments, reproduce or spread so aggressively that they harm their adopted ecosystems. Also called: exotic, alien, and non-indigenous species.

Latency: Delay between exposure to a disease-causing agent and manifestation of the disease (onset of infectiousness).

Lichen: Symbiotic associations between fungi and photosynthetic partners (algae).

Macrophage: Phagocytic cell derived from blood monocytes, typically resident in most tissues. It has both scavenger and antigen-presenting functions in immune responses.

Meiosis: The process in cell division in sexually reproducing organisms that reduces the number of chromosomes from diploid to haploid (half the original number).

Mendelian: A single gene disorder caused by a defect in one particular gene, and characterized by how they are passed down in families.

Metabolites: A substance produced by the chemical processes by which cells produce the substances and energy needed to sustain life.

Microbe: A microorganism or biologic agent that can replicate in humans (including bacteria, viruses, protozoa, fungi, and prions).

Microbial flora: The microscopic organisms living within a particular region, including bodily organ or body part, such as the skin.

Microbial threat: Microbes that lead to disease in humans.

Microbiology: A branch of biology dealing especially with microscopic forms of life.

Microbiome: Term used to describe the collective genome of our indigenous microbes (microflora).

Migration: The regular, usually seasonal, movement of all or part of an animal population to and from a given area.

Mitigation: Initiatives that reduce the risk from natural and man-made hazards.

Morbidity: Diseased condition or state.

Mortality: Proportion of deaths to population or to a specific number of the population; death rate.

Mutation: Genetic change that can occur either randomly or at an accelerated rate through exposure to radiation or certain chemicals (mutagens) and may lead to change in structure of the protein coded by the mutated gene.

Mycelia, mycelium: The mass of fine branching tubes (known as hyphae) that forms the main growing structure of a fungus.

Mycorrhizal fungi: Fungi that colonize plant roots.

Natural history: The natural development of something (such as an organism or disease) over a period of time.

Necropsy: An autopsy performed on an animal.

Neutrophil: Most common blood leukocyte; a short-lived phagocytic cell of the myeloid series, which is responsible for the primary cellular response to an acute inflammatory episode, and for general tissue homeostasis by removal of damaged material.

Notifiable disease: Disease that health professionals are required to report to state, national, or international authorities.

Obligate: Capable of existing only in a particular environment; an obligate parasite cannot survive independently of its host.

Old World: Refers to the Western Hemisphere; in a biological context: New World species are those from the Nearctic and Neotropic ecological zones; Old World species are those from the Palearctic and Afrotropic ecological zones.

One Health: Holistic approach to preventing epizootic disease and for maintaining ecosystem integrity for the benefit of humans, their domesticated animals, and the foundation biodiversity that supports all life.

Oomycete: Not a “true fungi”; an oomycete or “water mold” that belongs to the Kingdom Stramenopila (a major eukaryotic group that includes diatoms and brown algae, and is distinct from plants, fungi, and animals). Like fungi, oomycetes “exhibit filamentous growth, produce sexual and asexual spores, and can feed on decaying matter or be obligate parasites of plants.”

Opportunistic: Resulting from pathogen entry via wounds or weakened state of the host, or as a disturbance of a normally benign host–fungus relationship.

Pandemic: Disease outbreak occurring over a wide geographic area and affecting an exceptionally high proportion of the population.

Parasite, parasitic: An organism that lives in or on and takes its nourishment from another organism. A parasite cannot live independently. Parasitic diseases include infections by protozoa, helminths, and arthropods.

Pathogen: Organism capable of causing disease.

Pathogenic: Capable of causing disease.

Pathogenicity: The ability of an organism, a pathogen, to produce an infectious disease in another organism.

Pathology: The branch of medicine concerned with disease, especially its structure and its functional effects on the body.

Phagocyte/phagocytic cell: A cell that is capable of phagocytosis, or the uptake of particulate material by a cell. The main mammalian phagocytes are neutrophils and macrophages.

Phenology: The scientific study of cyclical biological events, such as flowering, breeding, and migration.

Phenotype: The physical appearance of an organism as distinguished from its genetic makeup (genotype).

Phylogeny: The connections among all groups of organisms as understood by ancestor/descendant relationships.

Phylum: In taxonomy and systematics: the highest level of classification below the kingdom.

Prevalence: Total number of cases (new as well as previous cases) of a disease in a given population at a point in time.

Primary fungal pathogen: Pathogens able to induce symptoms of disease in otherwise healthy individuals.

Propagules: Any of various structures that can give rise to a new individual organism. (For fungi, propagules include spores or encapsulated yeast cells.)

Psychotropic: Affecting mental activity, behavior, or perception.

Public health: The art and science of dealing with the protection and improvement of community health by organized community effort and including preventive medicine and sanitary and social health.

Quarantine: The enforced isolation or restriction of free movement imposed to prevent the spread of a contagious disease.

Race (plant pathology): A subspecies group of pathogens that infect a given set of plant varieties.

Recombination: A combining of genes or characters different from what they were in the parents.

Recombine: The process by which the combination of genes in an organism's offspring becomes different from the combination of genes in that organism.

Reservoir: Any person, animal, arthropod, plant, soil, or substance (or combination of these) in which an infectious agent normally lives and multiplies, on which it depends primarily for survival, and in which it reproduces itself in such manner that it can be transmitted to a susceptible vector or host.

Saprophytic: Deriving nutrients from dead organic matter.

Serotype: The characterization of a microorganism based on the kinds and combinations of constituent antigens (a substance that stimulates the production of an antibody when introduced into the body) present in that organism; a taxonomic subdivision of bacteria based on the above.

Somatic cells: The cells of the body, with the exception of the reproductive cells (gametes).

Species: The basic unit of taxonomy. A species is defined as a group of individuals that are genetically related and can interbreed to produce fertile young of the same kind.

Species barrier: Difficulty or impossibility for an infectious agent to pass from one species to another (due to differences between species).

Spores: Well-protected structures that can survive in adverse environmental conditions, such as freezing or drying (better than mycelia and yeast cells), for months and even years.

Surveillance: The continual scrutiny of all aspects of occurrence and spread of a disease that are pertinent to effective control, involves the systematic collection, analysis, interpretation, and dissemination of health data.

Symbiotic: The close association between two or more organisms of different species, often but not necessarily benefiting each member. The association of algae and fungi in lichens and of bacteria living in the intestines or on the skin of animals are symbiotic.

Syndrome: A group or recognizable pattern of symptoms or abnormalities that indicate a particular trait or disease.

Systematics: The classification of organisms and the evolutionary relationships among them; taxonomy.

Thermotolerance: Garcia Solache and Casadevall (2010) define thermotolerance as the ability to grow at mammalian (37°C) and higher temperatures. Most fungi thrive in the range of 12°C to 30°C, but there are wide temperature tolerances among species, with some growing at temperatures as low as -10°C or as high as 65°C. [Garcia-Solache, M. A., and A. Casadevall. 2010. Hypothesis: global warming will bring new fungal diseases for mammals. *mBio* 1(1):1–3.]

Transformation: The genetic alteration of a bacteria cell by the introduction of genetic material from another cell or from a virus.

Transmission: Process by which a pathogen passes from a source of infection to a new host.

Vaccine: A preparation of living, attenuated, or killed bacteria or viruses, fractions thereof, or synthesized or recombinant antigens identical or similar to those found in the disease-causing organism that is administered to raise immunity to a particular microorganism.

Vector: A carrier, especially an arthropod, that transfers an infective agent from one host (which can include itself) to another.

Vector borne: Transmitted from one host to another by a vector.

Virulence: The degree of pathogenicity of an organism as evidenced by the severity of resulting disease and the organism's ability to invade the host tissues.

Virulence factors: Molecules produced by a pathogen that specifically cause disease, or that influence their host's function to allow the pathogen to thrive.

Weather: The state of the atmosphere over a short period of time, measured in terms of wind, temperature, humidity, atmospheric pressure, cloudiness, and precipitation. The difference between weather and climate is a measure of time: Climate is how the atmosphere "behaves" over relatively long periods of time.

Xylose: A white crystalline sugar extracted from wood, straw, and corn.

Yeast: Any of various one-celled fungi that reproduce by budding and can cause the fermentation of carbohydrates, producing carbon dioxide and ethanol.

Zoonotic infection: Infection that causes disease in human populations but that can be perpetuated solely in non-human host animals (e.g., bubonic plague); may be enzootic or epizootic.

Appendix E

Forum Member Biographies

David A. Relman, M.D. (*Chair*), is the Thomas C. and Joan M. Merigan Professor in the Departments of Medicine and of Microbiology and Immunology at Stanford University, and Chief of Infectious Diseases at the VA Palo Alto Health Care System in Palo Alto, California. He received an S.B. (biology) from Massachusetts Institute of Technology (1977), received his M.D. (*magna cum laude*) from Harvard Medical School (1982), completed his clinical training in internal medicine and infectious diseases at Massachusetts General Hospital, served as a postdoctoral fellow in microbiology at Stanford University, and joined the faculty at Stanford in 1994.

Dr. Relman's current research focus is the human indigenous microbiota (microbiome) and, in particular, the nature and mechanisms of variation in patterns of microbial diversity within the human body as a function of time (microbial succession), space (biogeography within the host landscape), and in response to perturbation, for example, antibiotics (community robustness and resilience). One of the goals of this work is to define the role of the human microbiome in health and disease. This research integrates theory and methods from ecology, population biology, environmental microbiology, genomics, and clinical medicine. During the past few decades, his research directions have also included pathogen discovery and the development of new strategies for identifying previously unrecognized microbial agents of disease. This work helped to spearhead the application of molecular methods to the diagnosis of infectious diseases in the 1990s. His research has emphasized the use of genomic approaches for exploring host-microbe relationships. Past scientific achievements include the description of a novel approach for identifying previously unknown pathogens; the identification of a number of new human microbial pathogens, including the agent of

Whipple's disease; and some of the most extensive and revealing analyses to date of the human indigenous microbial ecosystem.

Dr. Relman advises the U.S. government, as well as nongovernmental organizations, in matters pertaining to microbiology, emerging infectious diseases, and biosecurity. He is a member of the National Science Advisory Board for Biosecurity, a member of the Physical and Life Sciences Directorate Review Committee for Lawrence Livermore National Laboratory, and he advises several U.S. government departments and agencies on matters related to pathogen diversity, the future life sciences landscape, and the nature of present and future biological threats. He has served as Chair of the Board of Scientific Counselors of the National Institute of Dental and Craniofacial Research (National Institutes of Health [NIH]) and as a member of the Board of Directors, Infectious Diseases Society of America (IDSA). Dr. Relman is currently vice-chair of a National Academy of Sciences (NAS) study of the science underlying the Federal Bureau of Investigation investigation of the 2001 anthrax mailings, and he cochaired a 3-year NAS study that produced a widely cited report entitled *Globalization, Biosecurity, and the Future of the Life Sciences* (2006). He is a Fellow of the American Academy of Microbiology and a member of the Association of American Physicians. Dr. Relman received the Squibb Award from the IDSA in 2001 and was the recipient of both the NIH Director's Pioneer Award and the Distinguished Clinical Scientist Award from the Doris Duke Charitable Foundation in 2006.

James M. Hughes, M.D. (Vice-Chair), is professor of medicine and public health at Emory University's School of Medicine and Rollins School of Public Health, serving as director of the Emory Program in Global Infectious Diseases, executive director of the Southeastern Center for Emerging Biologic Threats, and senior advisor to the Emory Center for Global Safe Water. He is the senior scientific advisor for infectious diseases to the International Association of National Public Health Institutes funded by the Bill and Melinda Gates Foundation. Prior to joining Emory in June 2005, Dr. Hughes served as director of the National Center for Infectious Diseases (NCID) at the Centers for Disease Control and Prevention (CDC). Dr. Hughes received his B.A. and M.D. degrees from Stanford University and completed postgraduate training in internal medicine at the University of Washington, infectious diseases at the University of Virginia, and preventive medicine at CDC. After joining CDC as an Epidemic Intelligence Service officer in 1973, Dr. Hughes worked initially on foodborne and water-related diseases and subsequently on infection control in health care settings. He served as director of CDC's Hospital Infections Program from 1983 to 1988, as deputy director of NCID from 1988 to 1992, and as director of NCID from 1992 to 2005. A major focus of Dr. Hughes' career is on building partnerships among the clinical, research, public health, and veterinary communities to prevent, detect, and respond to infectious diseases at the local, national and global levels. His research interests include emerging and reemerging infectious diseases, antimicrobial

resistance, foodborne diseases, health care-associated infections, vectorborne and zoonotic diseases, rapid detection of and response to infectious diseases and bioterrorism, strengthening public health capacity at the local, national, and global levels, and prevention of water-related diseases in the developing world. Dr. Hughes is a fellow and Council Delegate of the American Association for the Advancement of Science, fellow of the American College of Physicians and the Infectious Diseases Society of America (IDSA), President of IDSA, Councilor of the American Society of Tropical Medicine and Hygiene, and member of the International Board of the American Society for Microbiology. He is a member of the Institute of Medicine.

Lonnie J. King, D.V.M. (Vice-Chair), is the 10th dean of the College of Veterinary Medicine at the Ohio State University (OSU). In addition to leading this college, Dr. King is also a professor of preventive medicine and holds the Ruth Stanton Endowed Chair in Veterinary Medicine. Before becoming dean at OSU, he was the director of CDC's new National Center for Zoonotic, Vector-Borne, and Enteric Diseases (NCZVED). In this new position, Dr. King leads the Center's activities for surveillance, diagnostics, disease investigations, epidemiology, research, public education, policy development, and disease prevention and control programs. NCZVED also focuses on waterborne, foodborne, vectorborne, and zoonotic diseases of public health concern, which also include most of CDC's select and bioterrorism agents, neglected tropical diseases, and emerging zoonoses. Before serving as director, he was the first chief of the agency's Office of Strategy and Innovation.

Dr. King served as dean of the College of Veterinary Medicine, Michigan State University, from 1996 to 2006. As at OSU, he served as the CEO for academic programs, research, the teaching hospital, the diagnostic center for population and animal health, basic and clinical science departments, and the outreach and continuing education programs. As dean and professor of large-animal clinical sciences, Dr. King was instrumental in obtaining funds for the construction of a \$60 million Diagnostic Center for Population and Animal Health; he initiated the Center for Emerging Infectious Diseases in the college, he served as the campus leader in food safety, and he had oversight for the National Food Safety and Toxicology Center.

In 1992, Dr. King was appointed administrator for the Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), in Washington, DC. In this role, he provided executive leadership and direction for ensuring the health and care of animals and plants, to improve agricultural productivity and competitiveness, and to contribute to the national economy and public health. Dr. King also served as the country's chief veterinary officer for 5 years, worked extensively in global trade agreements within the North American Free Trade Agreement and the World Trade Organization, and worked extensively with the World Animal Health Association. During this time he was the Deputy

Administrator for Veterinary Services of APHIS, USDA, where he led national efforts in disease eradication, imports and exports, and diagnostics in both Ames, Iowa, and Plum Island. He spent 5 years in Hyattsville, Maryland, in staff assignments in Emergency Programs, as well as Animal Health Information. While in Hyattsville, Dr. King directed the development of the agency's National Animal Health Monitoring System. He left APHIS briefly to serve as the director of the Governmental Relations Division of the American Veterinary Medical Association (AVMA) in Washington, DC, and served as the lobbyist for the AVMA on Capitol Hill.

Dr. King was in private veterinary practice for 7 years in Dayton, Ohio, and Atlanta, Georgia. As a native of Wooster, Ohio, Dr. King received his bachelor of science and doctor of veterinary medicine degrees from OSU in 1966 and 1970, respectively. He earned his master of science degree in epidemiology from the University of Minnesota and received his master's degree in public administration from American University in Washington, DC, in 1991. Dr. King is a board-certified member of the American College of Veterinary Preventive Medicine and has completed the Senior Executive Fellowship program at Harvard University. He served as president of the Association of American Veterinary Medical Colleges from 1999 to 2000 and was the vice-chair for the National Commission on Veterinary Economic Issues from 2000 to 2004. He has served on four NAS committees, including chairing the National Academies' Committee on Assessing the Nation's Framework for Addressing Animal Diseases. He is also Chair of the IOM Committee on Lyme Disease and Other Tick-Borne Diseases and for State of the Science, and he is also chairing the AVMA's Commission for AVMA Vision 2020. Dr. King is currently a member of the IOM Committee on Microbial Threats to Health, is a past member of the Food and Drug Administration's (FDA's) Board of Scientific Advisors, and is past president of the American Veterinary Epidemiology Society. He served as the chair for the national One Medicine Task Force for the AVMA, which helped start the country's One Health Initiative. Dr. King was elected as a member of the IOM of the National Academies in 2004.

Kevin Anderson, Ph.D., serves as a Senior Program Manager in the Department of Homeland Security's Science and Technology Directorate, providing oversight and requirements for science programs focused on rapid detection and characterization of biological agents. Since joining DHS in 2003, Dr. Anderson has provided leadership for science program development, laboratory design and strategic planning, served as a subject matter expert and advisor to the Bioterrorism Risk Assessment and Biological Threat Characterization programs, and has participated in interagency working groups and assessments which provide guidance to medical countermeasure development, a key component of the nation's biodefense strategy. Prior to joining DHS, Dr. Anderson was a Principal Investigator at the U.S. Army Medical Research Institute of Infectious Diseases,

leading research focused on understanding basic mechanisms of viral diseases causing hemorrhagic fever and development of medical countermeasures. He received postdoctoral training in molecular virology at the University of Alabama at Birmingham and the University of North Carolina at Chapel Hill, performing basic research on human respiratory syncytial viruses, and earned Ph.D. and B.S. degrees in microbiology from Montana State University and the University of Maryland, College Park, respectively.

Ruth L. Berkelman, M.D., is the Rollins Professor and director of the Center for Public Health Preparedness and Research at the Rollins School of Public Health, Emory University, in Atlanta. She received her A.B. from Princeton University and her M.D. from Harvard Medical School. Board certified in pediatrics and internal medicine, she began her career at CDC in 1980 and later became deputy director of NCID. She also served as a senior advisor to the director of CDC and as assistant surgeon general in the U.S. Public Health Service. In 2001 she came to her current position at Emory University, directing a center focused on emerging infectious diseases and other urgent threats to health, including terrorism. She has also consulted with the biologic program of the Nuclear Threat Initiative and is most recognized for her work in infectious diseases and disease surveillance. She was elected to the IOM in 2004. Currently a member of the Board on Life Sciences of the National Academies, she also chairs the Board of Public and Scientific Affairs at the ASM.

David L. Blazes, M.D., M.P.H.,¹ Commander David L. Blazes is the Chief of Global Emerging Infections Surveillance and Response System (GEIS) Division at the Armed Forces Health Surveillance Center in Silver Spring, Maryland. From 2004–2008, he was Director of the Emerging Infections Department at the Naval Medical Research Center Detachment (now NAMRU-6) in Lima, Peru. The AFHSC-GEIS network identified the first cases of the 2009 H1N1 pandemic as well as numerous other emerging infections that threaten public health around the world. He also serves on the faculty at the Uniformed Services University in Bethesda, Maryland and in International Health at the Johns Hopkins Bloomberg School of Public Health. He graduated from the U.S. Naval Academy in 1991 and the Johns Hopkins University School of Medicine in 1995 and completed his internal medicine and infectious diseases training at the National Naval Medical Center, the President's hospital in Bethesda. His main scientific interests are infectious diseases surveillance strategies in developing settings, optimizing outbreak response, public health capacity building and tropical medicine training. He has taught clinical tropical medicine at the Gorgas course within Universidad Peruana Cayetano Heredia, at the Johns Hopkins Summer Institute of Tropical Medicine and at the U.S. Military Tropical Medicine course in Bethesda.

¹ Forum member since September 1, 2011.

Enriqueta C. Bond, Ph.D., is president emeritus of the Burroughs Wellcome Fund. Dr. Bond is currently a partner in QE Philanthropic Advisors, LLC, an organization that provides consulting services to foundations and non-profits on matters of program, strategic planning and capacity development related to medical sciences, international health, and science and math K–12 education. She received her undergraduate degree from Wellesley College, her M.A. from the University of Virginia, and her Ph.D. in molecular biology and biochemical genetics from Georgetown University. She is a member of the IOM and a fellow of the AAAS. Dr. Bond chairs the Academies' Board on African Science Academy Development and serves on the NRC Committee on the Future of the Research University. She serves on the board and executive committee of the Hamner Institute, the board of the Health Effects Institute, the board of the James B. Hunt Jr. Institute for Educational Leadership and Policy, and the NIH Council of Councils. In addition Dr. Bond serves on a scientific advisory committee for the World Health Organization (WHO) Tropical Disease Research Program. Prior to being named president of the Burroughs Wellcome Fund in 1994, Dr. Bond served on the staff of the IOM beginning in 1979, becoming its executive officer in 1989.

Roger G. Breeze, BVMS, Ph.D., MRCVS, is currently Bio-Security Deputy Program Director, Global Security Directorate, Office of Strategic Outcomes, Lawrence Livermore National Laboratory and serves on the senior management team of the Defense Threat Reduction Agency's Chemical and Biological Defense Directorate. He received his veterinary degree in 1968 and his Ph.D. in veterinary pathology in 1973, both from the University of Glasgow, Scotland. He was engaged in teaching, diagnostic pathology, and research on respiratory and cardiovascular diseases at the University of Glasgow Veterinary School from 1968 to 1977 and at Washington State University College of Veterinary Medicine from 1977 to 1987, where he was professor and chair of the Department of Microbiology and Pathology. From 1984 to 1987 he was deputy director of the Washington Technology Center, the state's high-technology sciences initiative, based in the College of Engineering at the University of Washington. In 1987, he was appointed director of the USDA's Plum Island Animal Disease Center, a Biosafety Level 3 (BSL-3) facility for research and diagnosis of the world's most dangerous livestock diseases. In that role he initiated research into the genomic and functional genomic basis of disease pathogenesis, diagnosis, and control of livestock RNA and DNA virus infections. This work became the basis of U.S. defense against natural and deliberate infection with these agents and led to his involvement in the early 1990s in biological weapons defense and proliferation prevention. From 1995 to 1998, he was South Atlantic area director for USDA's Agricultural Research Service before going to Washington, DC, to establish biological weapons defense programs for USDA. He received the Distinguished Executive Award from President Clinton in 1998 for his work at Plum Island and in biodefense. Since 2004 he has been CEO of Centaur Science group where

his main commitment was to the Defense Threat Reduction Agency's Global Bioengagement Program.

Steven J. Brickner, Ph.D.,² is an independent consultant based in southeastern Connecticut. He received his Ph.D. in organic chemistry from Cornell University and completed an NIH postdoctoral research fellowship at the University of Wisconsin, Madison. Dr. Brickner is a synthetic organic/medicinal chemist with more than 25 years of research experience focused entirely on the discovery of novel antibacterial agents during his prior tenure at Upjohn, Pharmacia & Upjohn, and Pfizer. He is co-inventor of Zynox[®] (linezolid), an oxazolidinone recognized as the first member of an entirely new class of antibiotic to reach the market in the more than 35 years since the discovery of the first quinolone. Approved in 2000, linezolid has annual worldwide sales of more than US\$1 billion. He initiated the oxazolidinone research program at Upjohn and led the team that discovered linezolid and clinical candidates eperzolid and PNU-100480. While at Pfizer, he led the early development team that placed the oxazolidinone PNU-100480 into clinical trials, where it is being studied as a potential treatment for tuberculosis. Dr. Brickner received an honorary doctor of science degree from the University of Notre Dame in 2010, and he was a corecipient of the Pharmaceutical Research and Manufacturers of America 2007 Discoverers Award and the 2007 American Chemical Society Award for Team Innovation. He was named the 2002–2003 Outstanding Alumni Lecturer, College of Arts and Science, Miami University (Ohio). He is an inventor or co-inventor on 21 U.S. patents, has published more than 30 peer-reviewed scientific papers, and has given 25 invited speaker presentations; he has been a member of the IOM Forum on Microbial Threats since 1997. In February 2009, he established SJ Brickner Consulting, LLC, which serves various clients in offering consulting services on all aspects of medicinal chemistry and drug design related to the discovery and development of new antibiotics.

Paula R. Bryant, Ph.D., is Chief of the Medical S&T Division, Chemical and Biological Defense Program at the Defense Threat Reduction Agency (DTRA) in Fort Belvoir, Virginia. As the Chief of the Medical S&T Division, Bryant interfaces with all levels of the Department of Defense and DTRA to plan, coordinate, integrate, and execute program activities necessary to provide timely and effective medical countermeasures against Chemical, Biological and Radiological (CBR) threats to U.S. interests worldwide. She also served as a Senior Scientist and Senior S&T Manager while at DTRA. Prior to joining DTRA, she was an Assistant Professor in the Department of Microbiology at The Ohio State University. She received her Ph.D. in Microbiology and Immunology from the Baylor College of Medicine.

² Forum member until December 31, 2010.

John E. Burris, Ph.D., became president of the Burroughs Wellcome Fund in July 2008. He is the former president of Beloit College. Prior to his appointment at Beloit in 2000, Dr. Burris served for 8 years as director and CEO of the Marine Biological Laboratory in Woods Hole, Massachusetts. From 1984 to 1992 he was at the National Research Council/National Academies, where he served as the executive director of the Commission on Life Sciences. A native of Wisconsin, he received an A.B. in biology from Harvard University in 1971, attended the University of Wisconsin, Madison, in an M.D.-Ph.D. program, and received a Ph.D. in marine biology from the Scripps Institution of Oceanography at the University of California, San Diego, in 1976. A professor of biology at the Pennsylvania State University from 1976 to 1985, he held an adjunct appointment there until going to Beloit. His research interests are in the areas of marine and terrestrial plant physiology and ecology. He has served as president of the American Institute of Biological Sciences and is or has been a member of a number of distinguished scientific boards and advisory committees, including the Grass Foundation; the Stazione Zoologica “Anton Dohrn” in Naples, Italy; the AAAS; and the Radiation Effects Research Foundation in Hiroshima, Japan. He has also served as a consultant to the National Conference of Catholic Bishops’ Committee on Science and Human Values.

Arturo Casadevall, M.D., Ph.D.,³ is the Leo and Julia Forchheimer Professor of Microbiology & Immunology at the Albert Einstein College of Medicine of Yeshiva University in the Bronx, New York. He is Chairman of the Department of Microbiology and Immunology and served as Director of the Division of Infectious Diseases at the Montefiore Medical Center at the Albert Einstein College of Medicine from 2000–2006. Dr. Casadevall received both his M.D. and Ph.D. (biochemistry) degrees from New York University in New York, New York. Subsequently, he completed internship and residency in internal medicine at Bellevue Hospital in New York, New York. Later he completed subspecialty training in Infectious Diseases at the Montefiore Medical Center and Albert Einstein College of Medicine. Dr. Casadevall major research interests are in fungal pathogenesis and the mechanism of antibody action. In the area of Biodefense Dr. Casadevall has an active research program to understand the mechanisms of antibody-mediated neutralization of *Bacillus anthracis* toxins. He has authored over 500 scientific papers. Dr. Casadevall was elected to membership in the American Society for Clinical Investigation, the American Academy of Physicians, and the American Academy of Microbiology. He was elected a fellow of the American Academy for the Advancement of Science and has received numerous honors including the Solomon A Berson Medical Alumni Achievement Award in Basic Science from the NYU School of Medicine, the Maxwell L. Littman Award

³ Forum member since September 1, 2011.

(mycology award), the Rhoda Benham Award from Medical Mycology Society of America, and the Kass Lecture of the Infectious Disease Society of America. Dr. Casadevall is the Editor in Chief of mBio, the first open access general journal of the American Society of Microbiology. He serves in the editorial board of the Journal of Clinical Investigation, The Journal of Experimental Medicine and The Journal of Infectious Diseases. Previously he served as Editor of Infection and Immunity. He has served in numerous NIH committees including those that drafted the NIAID Strategic Plan and the Blue Ribbon Panel on Biodefense Research. Dr. Casadevall served on the NAS committee that reviewed the science behind the FBI investigation of the anthrax attacks in 2001. He is currently a member of the National Science Advisory Board for Biosecurity and co-chaired the NIAID Board of Scientific counselors.

Since he joined the Einstein faculty in 1992 Dr. Casadevall has mentored dozens of graduate students, postdoctoral fellows, and junior faculty. Many of his trainees have gone on to have highly successful careers in science and several have currently AECOM faculty. From 2000–2006 Dr. Casadevall was director of the Division of Infectious Diseases at AECOM-Montefiore and oversaw the expansion of its research program. In 2001 Dr. Casadevall received the Samuel M. Rosen outstanding teacher award and in 2008 he was recognized the American Society of Microbiology with the William Hinton Award for mentoring scientists from underrepresented groups.

Peter Daszak, Ph.D., is President of EcoHealth Alliance (formerly Wildlife Trust), a U.S.-based organization which conducts research and field programs on global health and conservation. At Wildlife Trust, Dr. Daszak manages a headquarters staff of 35 and a global staff of more than 700 which conducts research and manages initiatives to prevent emerging pandemics and conserve wildlife biodiversity. This includes research on zoonoses that spill over from wildlife in emerging disease “hotspots,” including influenza, Nipah virus, SARS, West Nile virus, and others. Dr. Daszak’s work includes identifying the first case of a species extinction due to disease, the discovery of chytridiomycosis, the major cause of global amphibian declines, publishing the first paper to highlight emerging diseases of wildlife, coining the term “pathogen pollution,” discovery of the bat origin of SARS-like coronaviruses, identifying the drivers of Nipah and Hendra virus emergence, and producing the first ever emerging disease “hotspots” map.

Dr. Daszak is a member of the Council of Advisors of the One Health Commission, Treasurer of DIVERSITAS (ICSU), past member of the International Standing Advisory Board of the Australian Biosecurity CRC, past member of the IOM Committee on Global Surveillance for Emerging Zoonoses and the National Research Council (NRC) committee on the future of veterinary research. He is Editor-in-Chief of the Springer journal *Ecohealth*, and past treasurer and a founding director of the International Ecohealth Association. In 2000, he won the Commonwealth Scientific and Industrial Research Organisation medal for col-

laborative research in the discovery of amphibian chytridiomycosis. He has published over 130 scientific papers and book chapters, including papers in *Science*, *Nature*, *PNAS*, *The Lancet*, *PLoS Biology*, and other leading journals. His work has been the focus of articles in the *New York Times*, *The Wall Street Journal*, *The Economist*, *The Washington Post*, *US News & World Report*, *CBS 60 Minutes*, *CNN*, *ABC*, *NPR's Talk of the Nation*, and *Morning Edition & Fresh Air with Terri Gross*. He is a former guest worker at the CDC, where he assisted in the pathology activity during the 1999 Nipah virus outbreak. His work is funded by the John E. Fogarty International Center of NIH, the National Institute of Allergy and Infectious Diseases (NIAID), the National Science Foundation (NSF), the U.S. Agency for International Development (USAID), Google.org, Rockefeller, and other foundations. To date, his group is one of the few to have been awarded three prestigious NIH/NSF Ecology of Infectious Disease awards and is one of four partners to share a recent multi-million-dollar award from USAID ("PREDICT") with the goal of predicting and preventing the next emerging zoonotic disease.

Jeffrey Scott Duchin, M.D., is Chief of the Communicable Disease Epidemiology & Immunization Section for Public Health–Seattle & King County, Washington, and Professor of Medicine, Division of Infectious Diseases and Adjunct Professor in the School of Public Health and Community Medicine at the University of Washington.

Dr. Duchin trained in internal medicine at Thomas Jefferson University Hospital. He completed a fellowship in general internal medicine and emergency medicine at the Hospital of the University of Pennsylvania and infectious disease subspecialty training at the University of Washington. After several years on the faculty at the University of Pennsylvania, he joined the Centers for Disease Control and Prevention's (CDC's) Epidemic Intelligence Service program where he was assigned to the National Center for Infectious Diseases, and the CDC's Preventive Medicine Residency program. He worked for CDC as a medical epidemiologist in the Divisions of Tuberculosis Elimination and HIV/AIDS Special Studies Branch before assuming his current position.

Dr. Duchin is a member of the Institute of Medicine's (IOM's) Forum on Medical and Public Health Preparedness for Catastrophic Events and a current member of the Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (ACIP). He is a Fellow of the Infectious Disease Society of America (IDSA) and of the American College of Physicians; a member of the IDSA's National and Global Public Health Committee and Pandemic Influenza Task Force; and is past-Chair of the IDSA's Bioemergencies Task Force.

Dr. Duchin serves on the Editorial Board and Technical Advisory Group for Communicable Disease Alert and Response to Mass Gatherings for the World Health Organization and previously served as a member of the Department of Health and Human Services 2004 Tiger Team consulting with the Government of Greece on health preparations for the 2004 Olympics, in Athens, Greece.

Dr. Duchin's peer review publications and research interests focus on communicable diseases of public health significance, and he has authored text book chapters on the epidemiology of HIV/AIDS, bioterrorism, and outbreak investigations.

Jonathan Eisen, Ph.D., is a professor at the Genome Center at the University of California (UC), Davis, and holds appointments in the Department of Evolution and Ecology in the College of Biological Sciences and Medical Microbiology and Immunology in the School of Medicine.

His research focuses on the mechanisms underlying the origin of novelty (how new processes and functions originate). Most of his work involves the use of high-throughput DNA sequencing methods to characterize microbes and then the use and development of computational methods to analyze this type of data. In particular, his computational work has focused on integrating evolutionary analysis with genome analysis—so-called phylogenomics. Previously, he applied this phylogenomic approach to cultured organisms, such as those from extreme environments and those with key properties as they relate to evolution or global climate cycles. Currently he is using sequencing and phylogenomic methods to study microbes directly in their natural habitats (i.e., without culturing). In particular he focuses on how communities of microbes interact with each other or with plant and animal hosts to create new functions. Dr. Eisen is also coordinating one of the largest microbial genome sequencing projects to date—the “Genomic Encyclopedia of Bacteria and Archaea” being done at the Department of Energy (DOE) Joint Genome Institute, where he holds an Adjunct Appointment.

In addition to his research, Dr. Eisen is also a vocal advocate for “open access” to scientific publications and is the Academic Editor-in-Chief of *PLoS Biology*. He is also an active and award-winning blogger/microblogger (e.g., <http://phylogenomics.blogspot.com>, <http://twitter.com/phylogenomics>). Prior to moving to UC Davis he was on the faculty of The Institute for Genomic Research (TIGR) in Rockville, Maryland. He earned his Ph.D. in biological sciences from Stanford University, where he worked on the evolution of DNA repair processes in the lab of Philip C. Hanawalt and his undergraduate degree in biology from Harvard College.

Mark B. Feinberg, M.D., Ph.D., is vice president for medical affairs and policy in global vaccine and infectious diseases at Merck & Co., Inc., and is responsible for global efforts to implement vaccines to achieve the greatest health benefits, including efforts to expand access to new vaccines in the developing world. Dr. Feinberg received a bachelor's degree magna cum laude from the University of Pennsylvania in 1978 and his M.D. and Ph.D. degrees from Stanford University School of Medicine in 1987. His Ph.D. research at Stanford was supervised by Dr. Irving Weissman and included time spent studying the molecular biology of the human retroviruses—human T-cell lymphotropic virus, type I (HTLV-I) and HIV—as a visiting scientist in the laboratory of Dr. Robert Gallo at the National

Cancer Institute. From 1985 to 1986, Dr. Feinberg served as a project officer for the IOM Committee on a National Strategy for AIDS. After receiving his M.D. and Ph.D. degrees, Dr. Feinberg pursued postgraduate residency training in internal medicine at the Brigham and Women's Hospital of Harvard Medical School and postdoctoral fellowship research in the laboratory of Dr. David Baltimore at the Whitehead Institute for Biomedical Research. From 1991 to 1995, Dr. Feinberg was an assistant professor of medicine and microbiology and immunology at the University of California, San Francisco (UCSF), where he also served as an attending physician in the AIDS-oncology division and as director of the virology research laboratory at San Francisco General Hospital. From 1995 to 1997, Dr. Feinberg was a medical officer in the Office of AIDS Research in the Office of the Director of the NIH, the chair of the NIH Coordinating Committee on AIDS Etiology and Pathogenesis Research, and an attending physician at the NIH Clinical Center. During this period, he also served as executive secretary of the NIH Panel to Define Principles of Therapy of HIV Infection. Prior to joining Merck in 2004, Dr. Feinberg served as professor of medicine and microbiology and immunology at the Emory University School of Medicine, as an investigator at the Emory Vaccine Center, and as an attending physician at Grady Memorial Hospital. At UCSF and Emory, Dr. Feinberg and colleagues were engaged in the preclinical development and evaluation of novel vaccines for HIV and other infectious diseases and in basic research studies focused on revealing fundamental aspects of the pathogenesis of AIDS. Dr. Feinberg also founded and served as the medical director of the Hope Clinic of the Emory Vaccine Center—a clinical research facility devoted to the clinical evaluation of novel vaccines and to translational research studies of human immune system biology. In addition to his other professional roles, Dr. Feinberg has also served as a consultant to, and a member of, several IOM and NAS committees. Dr. Feinberg currently serves as a member of the National Vaccine Advisory Committee and is a member of the Board of Trustees of the National Foundation for Infectious Diseases. Dr. Feinberg has earned board certification in internal medicine; he is a fellow of the American College of Physicians, a member of the Association of American Physicians, and the recipient of an Elizabeth Glaser Scientist Award from the Pediatric AIDS Foundation and an Innovation in Clinical Research Award from the Doris Duke Charitable Foundation.

Jacqueline Fletcher, Ph.D., Regents Professor of Plant Pathology at Oklahoma State University, received a B.S. in biology from Emory University, Atlanta, Georgia, an M.S. in botany from the University of Montana, and a Ph.D. in plant pathology from Texas A&M University. She served as a postdoctoral associate at the University of Illinois before joining OSU in 1984, where she was appointed Sarkeys Distinguished Professor in 2001 and Regents Professor in 2008. She was named a Fellow of the American Phytopathological Society (APS) in 2005 and a Fellow of AAAS in 2007.

Dr. Fletcher is Director of the National Institute for Microbial Forensics and Food and Agricultural Biosecurity (NIMFFAB), a multidisciplinary OSU initiative that addresses high-priority national issues in research, teaching/education, and outreach with emphases in microbial forensics applications in plant pathology and produce safety. The NIMFFAB serves as a spoke laboratory for the Department of Homeland Security (DHS)-affiliated National Bioforensic Analysis Center, in the area of plant pathogen forensics. Dr. Fletcher's research focuses on mechanisms of virulence and insect transmission of plant pathogenic bacteria; on the relationships between human pathogens, such as *Salmonella* and *Escherichia coli*, and plants; and on the emerging disciplines of microbial forensics and agricultural biosecurity.

Dr. Fletcher served on the APS Council for 10 years, including the 4-year APS presidential sequence. In the months following September 11, 2001, Dr. Fletcher led APS responses and input to new national biosecurity initiatives. She has served for 9 years on the APS Public Policy Board (4 years as chair) and is currently on the APS Threatening Pathogens Advisory Committee. She also serves on several federal biosecurity advisory panels.

S. Elizabeth George, Ph.D.,⁴ is director of the Biological Countermeasures Portfolio within the Science and Technology Directorate in DHS. Until it merged into the new department in 2003, she was Program Manager of the Chemical and Biological National Security Program in DOE's National Nuclear Security Administration's Office of Nonproliferation Research and Engineering. Significant accomplishments include the design and deployment of BioWatch, the nation's first civilian biological threat agent monitoring system, and PROTECT, the first civilian operational chemical detection and response capability deployed in the Washington, DC, area subway system. Previously, she spent 16 years at the U.S. Environmental Protection Agency (EPA), Office of Research and Development, National Health and Ecological Effects Research Laboratory, Environmental Carcinogenesis Division, where she was Branch Chief of the Molecular and Cellular Toxicology Branch. She received her B.S. in biology in 1977 from Virginia Polytechnic Institute and State University and her M.S. and Ph.D. in microbiology in 1979 and 1984, respectively, from North Carolina State University. From 1984 to 1986, she was an NRC fellow in the laboratory of Dr. Larry Claxton at EPA. Dr. George is the 2005 chair of the Chemical and Biological Terrorism Defense Gordon Research Conference. She has served as Councillor for the Environmental Mutagen Society and President and Secretary of the Genotoxicity and Environmental Mutagen Society. She holds memberships in the ASM and the AAAS and is an adjunct faculty member in the School of Rural Public Health, Texas A&M University. She is a recipient of the EPA Bronze Medal and Scientific and Technological Achievement Awards and the Department of Home-

⁴ Forum member until December 31, 2010.

land Security Under Secretary's Award for Science and Technology. She is the author of numerous journal articles and has presented her research at national and international meetings.

Jesse L. Goodman, M.D., M.P.H., became chief scientist and deputy commissioner for science and public health of FDA in 2009. He has broad responsibility for and engagement in leadership and coordination of FDA's crosscutting scientific and public health efforts. From 2003 to 2009, Dr. Goodman was director of FDA's Center for Biologics Evaluation and Research, which oversees medical and public health activities critical to U.S. and global preparedness and the development, evaluation, safety, quality, and availability of biologics. A graduate of Harvard, Dr. Goodman received his M.D. from the Albert Einstein College of Medicine and did residency and fellowship training at the Hospital of the University of Pennsylvania and at the University of California, Los Angeles (UCLA), where he was also Chief Medical Resident. Prior to joining FDA, he was professor of medicine and chief of infectious diseases at the University of Minnesota, where he directed the multihospital infectious diseases research, training, and clinical programs, and where his NIH-funded laboratory first isolated and characterized *Anaplasma phagocytophilum*, the infectious agent causing a new tickborne disease, human granulocytic ehrlichiosis. Dr. Goodman has authored numerous scientific papers and edited the book *Tick Borne Diseases of Humans* (ASM Press, 2005). Dr. Goodman has been elected to the American Society for Clinical Investigation and to the IOM of the NAS, where he is a longstanding member of the Forum on Microbial Threats. He is an active clinician and teacher who is board certified in internal medicine, oncology, and infectious diseases and is staff physician and infectious diseases consultant at the National Naval and Walter Reed Army Medical Centers. Dr. Goodman is adjunct professor of medicine at the University of Minnesota.

Eduardo Gotuzzo, M.D., is principal professor of the Department of Medicine and director of the "Alexander von Humboldt" Institute of Tropical Medicine and Infectious Diseases, Peruvian University Cayetano Heredia in Lima, Peru, and head of the Department of Transmissible Diseases at the Cayetano Heredia Hospital. He is also an adjunct professor of medicine at the University of Alabama, Birmingham, School of Medicine. He is director of the International Gorgas Course in Clinical Tropical Medicine, Universidad Peruana Cayetano Heredia, taught jointly with the University of Alabama, Birmingham. He is an adjunct faculty member of the William J. Harrington Training Programs for Latin America, University of Miami School of Medicine (since 1983); was associate to the International Health Department of the Johns Hopkins University (1986–1998); and was fellow of the Center for the Americas at Vanderbilt, Vanderbilt University. Dr. Gotuzzo is an active member in numerous international societies and has been president of the Latin American Society of Tropical Disease (2000–2003); the

IDSA Scientific Program (2000–2003); the International Organizing Committee of the International Congress of Infectious Diseases (1994 to present); the International Society for Infectious Diseases (1996–1998); the PanAmerican Infectious Diseases Association; the International Federation for Tropical Medicine (2005–2008); and president of the Peruvian Society of Internal Medicine (1991–1992). He works on several research areas and teaches on subjects including emerging diseases, TB, HTLV-1, free-living amoebas, brucellosis, and parasites. He has published more than 290 articles and chapters as well as six manuals and one book. Recent honors and awards include being named an honorary member of the American Society of Tropical Medicine and Hygiene in 2002; an honorary member of the Society of Internal Medicine in 2000; and a distinguished visitor at the Faculty of Medical Sciences, University of Cordoba, Argentina (1999). In 1988, Dr. Gotuzzo received the Golden Medal for Outstanding Contribution in the Field of Infectious Diseases awarded by Trnava University, Slovakia. In 2007, Dr. Gotuzzo received the Society Citation Award from the IDSA. He was an honorary member of the Australian Society for Infectious Diseases (2008), the American Society of Tropical Medicine and Hygiene (2002), Academia de Medicina de México, Sociedad Nenzolana de Infectología, Sociedad Paraguaya de Infectología, and the National Academy of Medicine of Mexico (2010).

Carole A. Heilman, Ph.D., serves as director of the Division of Microbiology and Infectious Diseases (DMID) of NIAID, a component of NIH. DMID supports research to prevent and control diseases caused by virtually all human infectious agents (except HIV), including bacterial, viral, parasitic, and prion diseases. DMID supports a wide variety of projects spanning the spectrum from basic biology of human pathogens and their interaction with human hosts, through translational and clinical research, toward the development of new and improved diagnostics, drugs, and vaccines for infectious diseases. As director, Dr. Heilman provides scientific direction, oversight, and management for an extramural research portfolio that encompasses 300 different organisms.

DMID supports the nation's biodefense as well as a solid research infrastructure that readily responds to public health challenges, such as emerging diseases. These resources were recently mobilized to respond to the emergence of 2009 H1N1 influenza by providing the first in-depth characterization of the H1N1 pandemic virus and conducting nine clinical trials that provided safety and efficacy data to inform public health practice.

Dr. Heilman has a Ph.D. in microbiology from Rutgers University. She did her postdoctoral work in molecular virology at the National Cancer Institute (NCI) and continued at the NCI as a senior staff fellow in molecular oncology. She later moved into health science administration, where she focused on respiratory pathogens, particularly vaccine development. Dr. Heilman has received numerous awards for scientific management and leadership, including three Department of Health and Human Services (HHS) Secretary's Awards

for Distinguished Service recognizing her efforts on development of acellular pertussis vaccines, AIDS vaccines, and on accelerating biodefense research and development (R&D). Dr. Heilman serves as an infectious disease expert on the Board of Scientific Counselors for CDC. She also serves on the scientific board of the Fondation Mérieux's annual Advanced Course of Vaccinology and is a lecturer in this highly selective training program for decision makers in vaccinology. Throughout her career, Dr. Heilman has been a pioneer supporting the advancement of women in biomedical careers and serves as a mentor to a number of women within and outside of NIAID.

David L. Heymann, M.D., is currently chair of the Health Protection Agency, United Kingdom; professor and chair, infectious disease epidemiology, at the London School of Hygiene and Tropical Medicine; and head of the Global Health Security Programme at Chatham House, London. Until April 2009, he was assistant director-general for Health Security Environment and Representative of the director-general for Polio Eradication at WHO. Prior to that, from July 1998 until July 2003, he was executive director of the WHO Communicable Diseases Cluster, which included WHO's programs on infectious and tropical diseases, and from which the public health response to severe acute respiratory syndrome (SARS) was mounted in 2003. From October 1995 to July 1998, he was director of the WHO Programme on Emerging and Other Communicable Diseases, and prior to that he was the chief of research activities in the WHO Global Programme on AIDS. Dr. Heymann has worked in the area of public health for the past 35 years, 25 of which were on various assignments from CDC, and 10 of which have been with WHO. Before joining WHO, Dr. Heymann worked for 13 years as a medical epidemiologist in sub-Saharan Africa (Cameroon, Côte d'Ivoire, Malawi, and the Democratic Republic of Congo, formerly Zaire) on assignment from CDC in CDC-supported activities. These activities aimed at strengthening capacity in surveillance of infectious diseases and their control, with special emphasis on the childhood immunizable diseases, including measles and polio, African hemorrhagic fevers, poxviruses, and malaria. While based in Africa, Dr. Heymann participated in the investigation of the first outbreak of Ebola in Yambuku (former Zaire) in 1976, then again investigated the second outbreak of Ebola in 1977 in Tandala, and in 1995 directed the international response to the Ebola outbreak in Kikwit for WHO. Prior to assignments in Africa, he was assigned for 2 years to India as a medical epidemiologist in the WHO Smallpox Eradication Programme. Dr. Heymann's educational qualifications include a B.A. from the Pennsylvania State University, an M.D. from Wake Forest University, a Diploma in Tropical Medicine and Hygiene from the London School of Hygiene and Tropical Medicine, and practical epidemiology training in the 2-year Epidemic Intelligence Service of CDC. He is a member of the IOM; he was awarded the 2004 Award for Excellence of the American Public Health Association, the 2005 Donald Mackay Award from the American Society for Tropical Medicine and

Hygiene, and the 2007 Heinz Award on the Human Condition. In 2009 he was appointed an honorary Commander of the Most Excellent Order of the British Empire for services to global public health, and he was recently elected a Fellow of the Academy of Medical Sciences in the United Kingdom. Dr. Heymann has been visiting professor at Stanford University, the University of Southern California, and the George Washington University School of Public Health; has published over 145 scientific articles on infectious diseases and related issues in peer-reviewed medical and scientific journals; and has authored several chapters on infectious diseases in medical textbooks. He is currently the editor of the 19th edition of the *Control of Communicable Diseases Manual*, a joint publication of the American Public Health Association and WHO.

Philip Hosbach currently holds the position of vice president of immunization policy and government relations at sanofi pasteur. The departments under his supervision are state government affairs, federal government affairs, medical communications, strategic advocacy, and immunization initiatives. His responsibilities include oversight of both public policy and immunization policy development. Mr. Hosbach acts as sanofi pasteur's principal liaison with CDC. He is currently coordinating sanofi pasteur's global efforts in responding to the emerging H1N1 pandemic. He is a graduate of Lafayette College (1984); shortly after that he began his professional career in the pharmaceutical industry with American Home Products. That career has now spanned 25 years, including the last 22 years focused solely on vaccines. Mr. Hosbach joined sanofi pasteur (then Connaught Labs) in 1987 in Clinical Research and held positions of increasing responsibility, including Director of Clinical Operations. While in Clinical Research, he also served as project manager for the development and licensure of Tripedia, the first diphtheria, tetanus, and acellular pertussis vaccine approved by FDA for use in U.S. infants. During his clinical research career at sanofi pasteur, he contributed to the development and licensure of seven vaccines. Following his work in clinical research, Mr. Hosbach took a position in the commercial operations area of sanofi pasteur and quickly moved through the ranks on the business administration side of the vaccine division. During that time, Mr. Hosbach led a number of departments within sanofi pasteur, gaining valuable business experience within U.S. Commercial Operations. The departments he led during that time included Public Health Sales and Marketing, Public Relations, Public Affairs, New Product Marketing, and Business Intelligence. He has been a member of the IOM Forum on Microbial Threats since 2005 and has been a Steering Committee member of the Influenza Summit, which is jointly sponsored by the CDC and the American Medical Association, since its inception. Since 2000 Mr. Hosbach has served on the Board of Directors for Pocono Medical Center and Pocono Health Systems, located in East Stroudsburg, Pennsylvania. He also serves as chairman of the Compensation Committee.

Stephen Albert Johnston, Ph.D., is currently director of the Center for Innovations in Medicine in the Biodesign Institute at Arizona State University. His center focuses on formulating and implementing disruptive technologies for basic problems in health care. The center has three divisions: Genomes to Vaccines, Cancer Eradication, and DocInBox. Genomes to Vaccines has developed high-throughput systems to screen for vaccine candidates and is applying them to predict and produce chemical vaccines. The Cancer Eradication group is working on formulating a universal prophylactic vaccine for cancer. DocInBox is developing technologies to facilitate presymptomatic diagnosis. Dr. Johnston founded the Center for Biomedical Inventions (also known as the Center for Translation Research) at the University of Texas, Southwestern, the first center of its kind in the medical arena. He and his colleagues have developed numerous inventions and innovations, including the gene gun, genetic immunization, the tobacco etch virus protease system, organelle transformation, digital optical chemistry arrays, expression library immunization, linear expression elements, synbodies, immunosignaturing diagnosis, and others. He also was involved in transcription research for years, first cloning *Gal4* and later discovering functional domains in transcription factors and the connection of the proteasome to transcription. He has been professor at the University of Texas Southwestern Medical Center at Dallas and associate and assistant professor at Duke University. He has been involved in several capacities as an adviser on biosecurity since 1996 and is a founding member of BioChem 20/20.

Kent Kester, M.D., is currently the commander of the Walter Reed Army Institute of Research (WRAIR) in Silver Spring, Maryland. Dr. Kester holds an undergraduate biology degree from Bucknell University (1982) and an M.D. from Jefferson Medical College (1986). He completed his internship and residency in internal medicine at the University of Maryland Hospital/Baltimore VA Medical Center (1989) and a fellowship in infectious diseases at the Walter Reed Army Medical Center (1995). A malaria vaccine researcher with over 50 authored or coauthored scientific manuscripts and book chapters, Dr. Kester has played a major role in the development of the candidate falciparum malaria vaccine known as RTS,S, having safely conducted the largest number of experimental malaria challenge studies ever attempted to date. Dr. Kester's previous military medical research assignments have included director of the WRAIR Malaria Serology Reference Laboratory; chief, Clinical Malaria Vaccine Development Program; chief of the WRAIR Clinical Trials Center; and director of the WRAIR Division of Regulated Activities. He currently is a member of the Steering Committee of the NIAID/Uniformed Services University of the Health Sciences Infectious Disease Clinical Research Program, as well as multiple NIAID Safety Monitoring Committees. He also serves as the consultant to the U.S. Army Surgeon General in Medical Research and Development. Board certified in both internal medicine and infectious diseases, Dr. Kester is also a fellow of both the Ameri-

can College of Physicians and the IDSA. He holds faculty appointments at both the Uniformed Services University of the Health Sciences and the University of Maryland School of Medicine.

Gerald T. Keusch, M.D., is associate provost and associate dean for global health at Boston University and Boston University School of Public Health. He is a graduate of Columbia College (1958) and Harvard Medical School (1963). After completing a residency in internal medicine, fellowship training in infectious diseases, and 2 years as an NIH research associate at the Southeast Asia Treaty Organization Medical Research Laboratory in Bangkok, Thailand, Dr. Keusch joined the faculty of the Mt. Sinai School of Medicine in 1970, where he established a laboratory to study the pathogenesis of bacillary dysentery and the biology and biochemistry of Shiga toxin. In 1979 he moved to Tufts Medical School and New England Medical Center in Boston to found the Division of Geographic Medicine, which focused on the molecular and cellular biology of tropical infectious diseases. In 1986 he integrated the clinical infectious diseases program into the Division of Geographic Medicine and Infectious Diseases, continuing as division chief until 1998. He has worked in the laboratory and in the field in Latin America, Africa, and Asia on basic and clinical infectious diseases and HIV/AIDS research. From 1998 to 2003, he was associate director for international research and director of the Fogarty International Center at NIH. Dr. Keusch is a member of the American Society for Clinical Investigation, the Association of American Physicians, the ASM, and the IDSA. He has received the Squibb (1981), Finland (1997), and Bristol (2002) awards of the IDSA. In 2002 he was elected to the IOM.

Rima F. Khabbaz, M.D., is deputy director for infectious diseases at CDC. Prior to her current position, she served as director of CDC's National Center for Preparedness, Detection, and Control of Infectious Diseases and held other leadership positions across the agency's infectious disease national centers. She is a graduate of the American University of Beirut, Lebanon, where she obtained both her bachelor's degree in science and her medical doctorate degree. She trained in internal medicine and completed a fellowship in infectious diseases at the University of Maryland, Baltimore. She joined CDC in 1980 as an epidemic intelligence service officer, working in the Hospital Infections Program. During her CDC career, she has made major contributions to advance infectious disease prevention, including leadership in defining the epidemiology of non-HIV retroviruses (HTLV-I and II) in the United States and developing guidance for counseling HTLV-infected persons, establishing national surveillance for hantavirus pulmonary syndrome following the 1993 U.S. outbreak, and developing CDC's blood safety and food safety programs related to viral diseases. She has also played key roles in CDC's responses to outbreaks of new and/or reemerging viral infections, including Nipah, Ebola, West Nile, SARS, and monkeypox, as

well as the 2001 anthrax attacks. She is a fellow of the IDSA and member of the American Epidemiologic Society, the ASM, and the Council of State and Territorial Epidemiologists. She served on IDSA's Annual Meeting Scientific Program Committee and currently serves on the society's National and Global Public Health Committee. In addition to her CDC position, she serves as clinical associate professor of medicine (infectious diseases) at Emory University. She is a graduate of the National Preparedness Leadership Initiative at Harvard University and of the Public Health Leadership Institute at the University of North Carolina.

Stanley M. Lemon, M.D., is professor of medicine at the University of North Carolina, School of Medicine, Chapel Hill, North Carolina. He received his undergraduate A.B. degree in biochemical sciences from Princeton University summa cum laude and his M.D. with honors from the University of Rochester. He completed postgraduate training in internal medicine and infectious diseases at the University of North Carolina, Chapel Hill, and is board certified in both. From 1977 to 1983 he served with the U.S. Army Medical Research and Development Command, followed by a 14-year period on the faculty of the University of North Carolina, School of Medicine. He moved to the University of Texas Medical Branch in 1997, serving first as chair of the Department of Microbiology and Immunology, then as dean of the School of Medicine from 1999 to 2004. Dr. Lemon's research interests relate to the molecular virology and pathogenesis of the positive-stranded RNA viruses responsible for hepatitis. He has had a long-standing interest in antiviral and vaccine development and has served as chair of FDA's Anti-Infective Drugs Advisory Committee. He is the past chair of the Steering Committee on Hepatitis and Poliomyelitis of the WHO Programme on Vaccine Development. He is past chair of the NCID-CDC Board of Scientific Counselors and currently serves as a member of the U.S. Delegation to the U.S.–Japan Cooperative Medical Sciences Program. He was co-chair of the NAS Committee on Advances in Technology and the Prevention of Their Application to Next Generation Biowarfare Threats, and he recently chaired an IOM study committee related to vaccines for the protection of the military against naturally occurring infectious disease threats.

Edward McSweegan, Ph.D., is a program officer at NIAID. He graduated from Boston College with a B.S. in biology in 1978. He has an M.S. in microbiology from the University of New Hampshire and a Ph.D. in microbiology from the University of Rhode Island. He was an NRC associate from 1984 to 1986 and did postdoctoral research at the Naval Medical Research Institute in Bethesda, Maryland. Dr. McSweegan served as an AAAS diplomacy fellow in the U.S. State Department from 1986 to 1988, where he helped to negotiate science and technology agreements with Poland, Hungary, and the former Soviet Union. After moving to NIH, he continued to work on international health and infectious disease projects in Egypt, Israel, India, and Russia. Currently, he manages

NIAID's bilateral program with India, the Indo-U.S. Vaccine Action Program, and he represents NIAID in the HHS Biotechnology Engagement Program with Russia and related countries. He is a member of AAAS, the ASM, and the National Association of Science Writers. He is the author of numerous journal and freelance articles.

Mark A. Miller, M.D., is currently the Director of the Division of International Epidemiology and Population Studies for the Fogarty International Center at the National Institutes of Health (NIH) in Bethesda, Maryland. He is also a Physician at the Yukon-Kuskokwim Delta Regional Hospital in Bethel, Alaska, which primarily serves Native Americans. He previously served as a Medical Officer on the Children's Vaccine Initiative for WHO and the CDC, and Medical Epidemiologist for the CDC National Immunizations Program and Epidemiology Program Office, Office of the Director. He also conducted research at the Armed Forces Research Institute for Medical Studies in Bangkok, Thailand, the Yale Arbovirus Research Unit, and Cornell University Medical College.

Dr. Miller received his B.A., magna cum laude, in neuroscience, biology, and human ecology from Amherst College in 1983, and his M.D. from Yale University School of Medicine in 1990. He completed his internal medicine residency at Yale New Haven Hospital/Hospital of St. Raphael and became board certified in 1994. He has served as a member of many professional societies and steering committees, including the Secretary's Advisory Council on Public Health Preparedness Smallpox Modeling and several NSF, HHS, and NIH task forces. He has presented and consulted nationally and internationally for organizations including USAID, the Pan American Health Organization, and the World Bank. Dr. Miller is a reviewer for nine journals, including the *Journal of Infectious Diseases*, *The Lancet*, and the *Journal of the American Public Health Association*. He has won many awards, including the Distinguished Service Medal, from the U.S. Public Health Service and the CDC. He has published more than 50 scientific articles in the peer-reviewed literature, nine books and/or book chapters, and more than 50 letters and abstracts.

Paul F. Miller, Ph.D.,⁵ is chief scientific officer for antibacterials research. He received his undergraduate degree in biology from LeMoyne College, and subsequently earned a Ph.D. in microbiology and immunology from the Albany Medical College in 1987. Following 4 years of postdoctoral studies on yeast molecular genetics at NIH in Bethesda, Maryland, Dr. Miller joined the Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company in Ann Arbor, Michigan, in 1990 as a senior scientist in the Infectious Diseases Department, where he developed a number of novel screens and mechanism-of-action

⁵ Forum member until July 31, 2011.

tools. He then moved to Pfizer in 1997 as manager of the Antibacterials Biology Research group within the Antibacterials, Immunology, and Cancer Zone at the Groton, Connecticut, research labs, and has taken on increasing responsibility since that time. In his current role, he is responsible for all antibacterial research activities through early clinical development, as well as collaboratively establishing R&D strategies in this disease area. His specific research interests and expertise include genetic mechanisms of intrinsic antibiotic resistance in bacteria as well as the use of novel genetic technologies for the elucidation of antibiotic mechanisms of action.

Stephen S. Morse, Ph.D.,⁶ is Professor of Epidemiology at the Mailman School of Public Health of Columbia University, and Director of the PREDICT project of the USAID Emerging Pandemic Threats program. He was also founding Director of the Columbia University Center for Public Health Preparedness. He returned to Columbia in 2000 after four years in government service as Program Manager at the Defense Advanced Research Projects Agency, where he codirected the Pathogen Countermeasures Program and subsequently directed the Advanced Diagnostics Program. Before going to Columbia, he was Assistant Professor of Virology at the Rockefeller University in New York, where he remains an adjunct faculty member. He is the editor of two books, *Emerging Viruses* (Oxford University Press, 1993; paperback, 1996), which was selected by *American Scientist* for its list of 100 Top Science Books of the 20th Century, and *The Evolutionary Biology of Viruses* (Raven Press, 1994). He was a founding Section Editor of the CDC journal *Emerging Infectious Diseases* and was formerly an Editor-in-Chief of the Pasteur Institute's journal *Research in Virology*. Dr. Morse was Chair and principal organizer of the 1989 NIAID-NIH Conference on Emerging Viruses, for which he originated the term and concept of emerging viruses/infections. He has served as a member of the IOM-NAS Committee on Emerging Microbial Threats to Health, chaired its Task Force on Viruses, and was a contributor to the resulting report *Emerging Infections* (1992). He has served on a number of NAS and IOM committees, including the IOM Committee on Xenograft Transplantation. Dr. Morse also served as an adviser to WHO and several government agencies. He is a fellow of the AAAS, the New York Academy of Sciences (and a past Chair of its microbiology section), the American Academy of Microbiology, the American College of Epidemiology, and an elected life member of the Council on Foreign Relations. He was the founding Chair of ProMED, the nonprofit international Program to Monitor Emerging Diseases, and was one of the originators of ProMED-mail, an international network inaugurated by ProMED in 1994 for outbreak reporting and disease monitoring using the Internet. Dr. Morse received his Ph.D. from the University of Wisconsin, Madison.

⁶ Forum member until December 31, 2010.

George Poste, Ph.D., D.V.M., is chief scientist, Complex Adaptive Systems Initiative, and Del E. Webb Professor of Health Innovation at Arizona State University (ASU). He assumed this post in 2009. From 2003 to 2009 he directed and built the Biodesign Institute at ASU. In addition to his academic post, he serves on the Board of Directors of Monsanto, Exelixis, Caris Life Sciences, LGC, and the Scientific Advisory Board of Synthetic Genomics. From 1992 to 1999 he was Chief Science and Technology Officer and President, R&D, of SmithKline Beecham (SB). During his tenure at SB he was associated with the successful registration of 31 drug, vaccine, and diagnostic products. In 2004 he was named “R&D Scientist of the Year” by *R&D Magazine*, in 2006 he received the Einstein award from the Global Business Leadership Council, and in 2009 he received the Scrip Lifetime Achievement award voted by the leadership of the global pharmaceutical industry.

He has published over 350 research papers and edited 14 books on pharmaceutical technologies and oncology. He has received honorary degrees in science, law, and medicine for his research contributions and was honored in 1999 by Her Majesty Queen Elizabeth II as a Commander of the British Empire for his contributions to international security. He is a Fellow of the Royal Society, the Royal College of Pathologists, and the U.K. Academy of Medicine; a Distinguished Fellow at the Hoover Institution, Stanford University; and a member of the Council on Foreign Relations. He has served on numerous government panels related to biosecurity and national competitiveness.

John C. Pottage, Jr., M.D., has been vice president for Global Clinical Development in the Infectious Disease Medicine Development Center at GlaxoSmith-Kline since 2007. Previously he was senior vice president and chief medical officer at Achillion Pharmaceuticals in New Haven, Connecticut. Achillion is a small biotechnology company devoted to the discovery and development of medicines for HIV, hepatitis C virus, and resistant antibiotics. Dr. Pottage initially joined Achillion in May 2002. Prior to Achillion, Dr. Pottage was medical director of Antivirals at Vertex Pharmaceuticals. During this time he also served as an associate attending physician at the Tufts New England Medical Center in Boston. From 1984 to 1998, Dr. Pottage was a faculty member at Rush Medical College in Chicago, where he held the position of associate professor, and also served as the medical director of the Outpatient HIV Clinic at Rush-Presbyterian-St. Luke’s Medical Center. While at Rush, Dr. Pottage was the recipient of several teaching awards and is a member of the Mark Lepper Society. Dr. Pottage is a graduate of St. Louis University School of Medicine and Colgate University.

David Rizzo⁷ received his Ph.D. in plant pathology from the University of Minnesota and joined the faculty of the University of California-Davis, Department

⁷ Forum member since September 1, 2011.

of Plant Pathology and the Graduate Group in Ecology in 1995. Research in his lab focuses on the ecology and management of forest tree diseases, including diseases caused by both native and introduced pathogens. Research in the lab takes a multiscale approach ranging from experimental studies on the basic biology of organisms to field studies across forest landscapes. Active collaborations include projects with landscape ecologists, epidemiologists, molecular biologists, entomologists, and forest managers. The primary research effort in the lab is currently *Phytophthora* species in California coastal forests, with an emphasis on Sudden Oak Death. As part of his research on Sudden oak Death, Dr. Rizzo also serves as the scientific advisor for the California Oak Mortality Task Force. In conifer forests of the Sierra Nevada Mountains, the lab studies a variety of diseases and their relationship to past and present forest management and conservation issues. In addition to research, Dr. Rizzo teaches undergraduate and graduate courses in mycology as well as introductory biology. Since 2004, he has been director of the Science and Society program in the College of Agricultural and Environmental Sciences. Science and Society is an academic program designed to offer students the opportunity to discover the interdisciplinary connections that link the biological, physical and social sciences with societal issues and cultural discourses.

Gary A. Roselle, M.D., is program director for infectious diseases for the VA Central Office in Washington, DC, as well as the chief of the medical service at the Cincinnati VA Medical Center. He is a professor of medicine in the Department of Internal Medicine, Division of Infectious Diseases, at the University of Cincinnati College Of Medicine. Dr. Roselle serves on several national advisory committees. In addition, he is currently heading the Emerging Pathogens Initiative for the VA. He has received commendations from the undersecretary for health for the VA and the secretary of VA for his work in the Infectious Diseases Program for the VA. He has been an invited speaker at several national and international meetings and has published more than 90 papers and several book chapters. Dr. Roselle received his medical degree from the OSU School of Medicine in 1973. He served his residency at the Northwestern University School of Medicine and his infectious diseases fellowship at the University of Cincinnati School of Medicine.

Alan S. Rudolph, Ph.D., M.B.A., has led an active career in translating interdisciplinary life sciences into useful applications for biotechnology development. His experience spans basic research to advanced development in academia, government laboratories, and most recently in the nonprofit and private sectors. He has published more than 100 technical publications in areas including molecular biophysics, lipid self-assembly, drug delivery, blood substitutes, medical imaging, tissue engineering, neuroscience, and diagnostics. As a National Research Council Post-Doctoral Fellow, his earliest work at the U.S. Naval Research Laboratory (NRL) demonstrated the translational value of strategies used by or-

ganisms that survive environmental extremes to preserve Defense products such as biosensors and blood products for field deployment. After a decade at NRL he was recruited to join the Defense Advanced Research Projects Agency, to lead new strategic efforts to extract and exploit useful principles and practices in life sciences and technology and establish an agency-wide strategy for investments in biosciences and biotechnology. As Chief of Biological Sciences and Technology, Dr. Rudolph established a framework for investments that continue today. These include new programs in broad areas of bioscience and technology such as sensors, diagnostics, materials, robotics, biomolecular, cell and tissue engineering, medical devices, and neuroscience and technology, including the current efforts in revolutionizing prosthetics. He received a meritorious civil service citation from the Office of the Secretary of Defense for his contributions to defining and implementing a new generation of life sciences and national security investments.

In 2003, he left civil service for the private sector and starting new corporate biotechnology efforts. As Chief Executive Officer of Adlyfe Inc., a diagnostic platform company, and Board Chairman of Cellphire Inc., focused on development of novel hemostatic biologics for bleeding injury, he took nascent technology demonstrations and secured venture capital funding and pharmaceutical partnerships while managing all aspects of development toward first human use. These efforts included managing early manufacturing and regulatory strategies required for FDA approval of diagnostics and therapeutics. Most recently, he started a new international nonprofit foundation and, as Director of the International Neuroscience Network Foundation, he has secured corporate and private philanthropic donors to fulfill the mission of the organization focused on brain STEM efforts and clinical trial management in underserved populations. He has a doctorate degree in zoology from the University of California at Davis and an M.B.A. from the George Washington University.

Kevin Russell, M.D., M.T.M.&H., F.I.D.S.A. CAPT MC USN, is the Director, Department of Defense Global Emerging Infections Surveillance and Response System, and Deputy Director, Armed Forces Health Surveillance Center, in the U.S. Department of Defense. In this position, his priorities have been standardization, greater affiliations with world militaries, continuing to introduce scientific rigor into the network, and synchronization with other U.S. government global surveillance programs. He graduated from the University of Texas Health Science Center San Antonio Medical School in 1990; after a family practice internship, he was accepted into the Navy Undersea Medicine program. He was stationed in Panama City, Florida, at the Experimental Diving Unit where he worked in diving medicine research from 1991 to 1995. After a preventive medicine residency with a masters in tropical medicine and hygiene, he was transferred to Lima, Peru, where he became head of the Virology Laboratory. His portfolio included febrile illness (largely arboviral in origin) and HIV surveillance studies in eight different countries of South America, as well as prospective dengue transmission studies.

In 2001, he moved back to the United States and became the director of the Respiratory Disease Laboratory at the Naval Health Research Center in San Diego, California. Febrile respiratory illness surveillance in recruits of all services was expanded into shipboard populations, Mexican border populations, support for outbreaks, and deployed settings. Validation and integration of new and emerging advanced diagnostic capabilities, utilizing the archives of specimens maintained at the laboratory, became a priority. A BSL-3-Enhanced was constructed. Projects expanded in 2006 to clinical trials support as Dr. Russell became the Principal Investigator for the Navy site in the FDA Phase III adenovirus vaccines trial, and more recently to support the Phase IV post-marketing trial of the recently FDA-approved ACAM2000 smallpox vaccine.

Janet Shoemaker is director of the ASM's Public Affairs Office, a position she has held since 1989. She is responsible for managing the legislative and regulatory affairs of this 42,000-member organization, the largest single biological science society in the world. Previously, she held positions as assistant director of public affairs for the ASM; as ASM coordinator of the U.S.–U.S.S.R. Exchange Program in Microbiology, a program sponsored and coordinated by the NSF and the U.S. Department of State; and as a freelance editor and writer. She received her baccalaureate, cum laude, from the University of Massachusetts and is a graduate of the George Washington University programs in public policy and in editing and publications. She is a member of Women in Government Relations, the American Society of Association Executives, and AAAS. She has coauthored articles on research funding, biotechnology, biodefense, and public policy issues related to microbiology.

P. Frederick Sparling, M.D., is professor of medicine, microbiology, and immunology at the University of North Carolina (UNC), Chapel Hill. He is director of the SouthEast Sexually Transmitted Infections Cooperative Research Center and also the Southeast Regional Centers of Excellence in Biodefense and Emerging Infections. Previously he served as chair of the Department of Medicine and chair of the Department of Microbiology and Immunology at UNC. He was president of the IDSA from 1996 to 1997. He was also a member of the IOM Committee on Microbial Threats to Health (1990–1992) and the IOM Committee on Emerging Microbial Threats to Health in the 21st Century (2001–2003). Dr. Sparling's laboratory research has been on the genetics and molecular biology of bacterial outer membrane proteins, with a major emphasis on gonococci and meningococci. His work helped to define the genetics of antibiotic resistance in gonococci and the role of iron-scavenging systems in the pathogenesis of human gonorrhea. Current interests include pathogenesis of gonococcal infections and development of a vaccine for gonorrhea and managing a large multi-institution interactive research group focused on emerging infections and biodefense.

Terence Taylor is the founding president of the International Council for the Life Sciences (ICLS). The ICLS is an independent nonprofit organization registered in the United States and in the European Union. The ICLS is designed to promote best practices and codes of conduct for safety and security in relation to biological risks. Terence Taylor also served as the vice president, Global Health and Security, at the Nuclear Threat Initiative. Prior to these appointments Terence Taylor was assistant director at the International Institute for Strategic Studies (IISS) in London and was president and executive director of IISS-US in Washington, DC. At IISS, in addition to his overall program responsibilities, he led the Institute's work on life sciences and security. He has substantial experience in international security policy matters as a U.K. government official (both military and diplomatic) and for the United Nations (UN) both in the field and at UN Headquarters. He was a commissioner and one of the Chief Inspectors with the UN Special Commission on Iraq, with particular responsibilities for biological issues. His government experience is related to both military field operations and to the development and implementation of policies in relation to arms control and nonproliferation treaties and agreements for both conventional weapons and weapons of mass destruction and the law of armed conflict aspects of International Humanitarian Law. He has also conducted consulting work on political risk assessment and studies of the private biotechnology industry. He was a Science Fellow at Stanford University's Center for International Security and Cooperation. He was an officer in the British Army with experience in many parts of the world including UN peacekeeping, counterinsurgency, and counterterrorism operations.

Murray Trostle, Dr.P.H., is a foreign service officer with USAID, presently serving as the deputy director of the Avian and Pandemic Influenza Preparedness and Response Unit. Dr. Trostle attended Yale University, where he received a master's in public health in 1978, focusing on health services administration. In 1990, he received his doctorate in public health from UCLA. His research involved household survival strategies during famine in Kenya. Dr. Trostle has worked in international health and development for approximately 38 years. He first worked overseas in the Malaysian national malaria eradication program in 1968 and has since focused on health development efforts in the former Soviet Union, Africa, and Southeast Asia. He began his career with USAID in 1992 as a postdoctoral fellow with AAAS. During his career he has worked with a number of development organizations, such as the American Red Cross, Project Concern International, and the Center for Development and Population Activities. With USAID, Dr. Trostle has served as director of the child immunization cluster, where he was chairman of the European Immunization Interagency Coordinating Committee and USAID representative to the Global Alliance on Vaccines and Immunization. Currently, Dr. Trostle leads the USAID Infectious Disease Surveillance Initiative as well as the Avian Influenza Unit.

Mary E. Wilson, M.D., is Associate Professor of Global Health and Population at the Harvard School of Public Health. Her academic interests include the ecology of infections and emergence of microbial threats, travel medicine, tuberculosis, and vaccines. Her undergraduate degree in French, English, and philosophy was awarded by Indiana University; she received her M.D. from the University of Wisconsin and completed an internal medicine residency and infectious disease fellowship at the Beth Israel Hospital in Boston (now Beth Israel-Deaconess Medical Center). She was Chief of Infectious Diseases at Mount Auburn Hospital, a Harvard-affiliated community teaching hospital in Cambridge, Massachusetts, for more than 20 years. She is a Fellow in the IDSA and the American College of Physicians. She has served on ACIP of the CDC, the Academic Advisory Committee for the National Institute of Public Health in Mexico, and on four committees for the IOM of the National Academies, including the Committee on Emerging Microbial Threats to Health in the 21st Century, whose report (*Microbial Threats to Health: Emergence, Detection, and Response*) was released in March 2003. She has worked in Haiti at the Albert Schweitzer Hospital and leads the Harvard-Brazil Collaborative Course on Infectious Diseases, which is taught in Brazil. In 1996 she was a resident scholar at the Bellagio Study Center, Italy, and in 2002 she was a Fellow at the Center for Advanced Study in the Behavioral Sciences in Stanford, California. She was a member of the Pew National Commission on Industrial Farm Animal Production, whose report *Putting Meat on the Table: Industrial Farm Animal Production in America* was released in the spring of 2008. A former GeoSentinel Site Director (Cambridge), she now serves as a Special Advisor to the GeoSentinel Surveillance Network, a global network. She has lectured and published widely, serves on several editorial boards, and is an associate editor for *Journal Watch Infectious Diseases*. She is the author of *A World Guide to Infections: Diseases, Distribution, Diagnosis* (Oxford University Press, New York, 1991); senior editor, with Richard Levins and Andrew Spielman, of *Disease in Evolution: Global Changes and Emergence of Infectious Diseases* (New York Academy of Sciences, 1994); and editor of the volume *New and Emerging Infectious Diseases* (Medical Clinics of North America) published in 2008. She joined the Board of Trustees for ICDDR, B (International Centre for Diarrheal Disease Research, Bangladesh) in 2009 and is a member of the Board of Scientific Counselors for the CDC, the FXB-USA Board, and the APUA Board of Directors.

Appendix F

Speaker Biographies

Karen Bartlett, Ph.D., M.Sc., received her B.A. from the University of Victoria, her M.Sc. in Occupational Hygiene from the University of British Columbia (UBC), and her Ph.D. in Interdisciplinary Studies (Environmental Health) also from UBC. She completed postdoctoral training in Inhalation Toxicology at the University of Iowa. She is an associate professor in the School of Environmental Health, in the College of Interdisciplinary Studies at UBC. Dr. Bartlett's current research interests are in four thematic areas: environmental sources of infectious disease; mold and building material interactions (the built environment); animal models of lung infections for therapeutic protocols; and occupational and environmental exposure to airborne biologic particles. Examples of recent research are bioaerosol exposures in the built environment (including First Nations housing); exposures to compost workers; and environmental sources of *Cryptococcus gattii*. Dr. Bartlett is an adjunct faculty in the Department of Pathology, UBC, and an associate faculty member in the Institute of Resource and Environmental Sustainability and the School of Populations and Public Health.

Meredith Blackwell, Ph.D., is interested in phylogeny, evolution, and life history studies of fungi associated with arthropods. Her current research focuses on the interactions between gut yeasts of fungus- and wood-feeding beetles. She and her colleagues have sampled yeasts from a largely unexplored habitat—the gut of beetles in the United States, Latin America, and Thailand—and discovered more than 200 undescribed species, about 20 percent of all known yeasts. Furthermore, gene cloning and imaging studies have led to the discovery of a larger community of gut organisms in wood-feeding beetles, including parabasalids and bacteria as well as the yeasts that produce enzymes that degrade plant cell walls.

Dr. Blackwell, who is Boyd Professor at Louisiana State University, has been involved in several projects involving the fungal systematics community. The recent Deep Hypha and Assembling the Fungal Tree of Life projects involved more than 100 mycologists from 25 countries in phylogenetic studies of major groups of fungi and a phylogenetic classification used in many major publications. Dr. Blackwell is a Distinguished Mycologist of the Mycological Society of America and Fellow of the British Mycological Society. She served as president of the International Mycological Association and the Mycological Society of America and is coauthor of *Introductory Mycology*, a widely used textbook currently under revision.

David Blehert, Ph.D., earned his doctorate degree in bacteriology in 1999 from the University of Wisconsin–Madison where he studied the biotransformation of munitions manufacturing wastes as mediated by soil bacteria. He then completed a postdoctoral fellowship at the National Institutes of Health in Bethesda, Maryland where he investigated bacterial communication mechanisms among constituents of the human dental plaque community. His research emphasis was on the role of the signaling molecule autoinducer-2 in the formation of bacterial biofilms. Dr. Blehert joined the U.S. Geological Survey–National Wildlife Health Center in Madison, Wisconsin, in 2003 as the head of the diagnostic microbiology laboratory. Current research projects under way in his laboratory include characterization of microbial aspects of the pathogenesis and epidemiology of bat white-nose syndrome; the use of molecular markers to understand the epidemiology of avian cholera in wild waterfowl; and development of rapid in vitro techniques for the detection of botulinum neurotoxins. His laboratory's collaborative efforts to identify the fungus that causes the skin infection hallmark of bat white-nose syndrome were published in the January 9, 2009, issue of *Science*.

Arturo Casadevall, M.D., Ph.D., is the Leo and Julia Forchheimer Professor and Chair of Microbiology & Immunology at the Albert Einstein College of Medicine. Dr. Casadevall received both his M.D. and Ph.D. (biochemistry) degrees from New York University in New York. Subsequently, he completed internship and residency in internal medicine at Bellevue Hospital in New York. Later he completed subspecialty training in infectious diseases. Dr. Casadevall's major research interests are in fungal pathogenesis and the mechanism of antibody action. Dr. Casadevall has authored more than 470 scientific papers. He has been elected to membership in the American Society for Clinical Investigation, American Academy of Physicians, and American Academy of Microbiology. He was elected a Fellow of the American Academy for the Advancement of Science (AAAS) and has received numerous honors. In 2005, he received the Alumni Award in basic science from his alma mater, New York University. Dr. Casadevall is editor in chief of *mBio* and serves on the editorial board of the *Journal of Clini-*

cal Investigation and the *Journal of Experimental Medicine*. In 2008, he received the Hinton Award from the American Society for Medicine for his efforts in training scientists from underrepresented minority groups.

Peter Daszak, Ph.D., is president of EcoHealth Alliance (formerly Wildlife Trust), a U.S.-based organization that conducts research and field programs on global health and conservation. At Wildlife Trust, Dr. Daszak manages a headquarters staff of 35 and a global staff of more than 700. The staff conduct research and manage initiatives to prevent emerging pandemics and to conserve wildlife biodiversity. This includes research on zoonoses that spill over from wildlife in emerging disease “hot spots,” including influenza, Nipah virus, Severe Acute Respiratory Syndrome (SARS), West Nile virus, and others. Dr. Daszak’s work includes identifying the first case of a species extinction due to disease; the discovery of chytridiomycosis, the major cause of global amphibian declines; publishing the first paper to highlight emerging diseases of wildlife; coining the term “pathogen pollution”; discovery of the bat origin of SARS-like coronaviruses; identifying the drivers of Nipah and Hendra virus emergence; and producing the first emerging disease “hot spots” map.

Dr. Daszak is a member of the Council of Advisors of the One Health Commission, Treasurer of DIVERSITAS, past member of the International Standing Advisory Board of the Australian Biosecurity CRC, and past member of the Institute of Medicine (IOM) Committee on Global Surveillance for Emerging Zoonoses and the National Research Council (NRC) committee on the future of veterinary research. He is editor in chief of the Springer journal *Ecohealth* and past treasurer and a founding director of the International Ecohealth Association. In 2000, he won the Commonwealth Scientific and Industrial Research Organisation medal for collaborative research in the discovery of amphibian chytridiomycosis. He has published more than 130 scientific papers and book chapters, including papers in *Science*, *Nature*, *PNAS*, *The Lancet*, *PLoS Biology*, and other leading journals. His work has been the focus of articles in the *New York Times*, *The Wall Street Journal*, *The Economist*, *The Washington Post*, *US News & World Report*, *CBS*, *60 Minutes*, *CNN*, *ABC*, *NPR’s Talk of the Nation*, and *Morning Edition & Fresh Air with Terri Gross*. He is a former guest worker at the Centers for Disease Control and Prevention (CDC), where he assisted in the pathology activity during the 1999 Nipah virus outbreak. His work is funded by the John E. Fogarty International Center of NIH, the National Institute of Allergy and Infectious Diseases, the National Science Foundation (NSF), the U.S. Agency for International Development (USAID), Google.org, Rockefeller, and other foundations. To date, his group is one of the few to have been awarded three prestigious NIH/NSF Ecology of Infectious Disease awards and is one of four partners to share a recent multimillion-dollar award from USAID (“PREDICT”) with the goal of predicting and preventing the next emerging zoonotic disease.

Matthew C. Fisher, Ph.D., currently holds the appointment of reader in the Department of Infectious Disease Epidemiology at the Imperial College London. He received his B.S. and Ph.D. in biology from Edinburgh University and was a Postdoctoral Fellow at the University of California, Berkeley.

Global health necessitates the adoption of a broad perspective: Anthropogenic activity is accelerating global changes, with inevitable decreases in human welfare as ecosystems deteriorate. His research program focuses on the kingdom Fungi, whose impact on global health is increasing as a consequence of global changes. Broadly Dr. Fisher's research investigates the changing impact of fungal disease by focusing on two themes: The first, fungal disease ecology, ascertains the environmental envelopes that are associated with mycoses, and the occurrence of fungal pandemics and panzootics. This theme has, at its core, the idea that anthropogenic activity is widely mixing fungal pathogens across global scales. However, the manifestation of disease only occurs if a match occurs between the invader-fungus and the recipient-host/biome: He investigates this within current and future-climate scenarios for several important human, animal, and plant pathogens. The second theme is fungal evolution, and here he investigates the adaptive stored potential that exists within different fungal pathogens. Dr. Fisher's combined use of population genetics, comparative genomics, and molecular epidemiology are used to decipher the evolutionary histories of mycoses, to investigate their origins, and to predict their future trajectories as pathogens.

John N. Galgiani, M.D., was born in San Francisco, received his B.A. from Stanford University, his M.D. from Northwestern University, and a fellowship in Infectious Diseases from Stanford. In 1978, Dr. Galgiani joined the faculty of the University of Arizona currently he is Professor of Medicine. Dr. Galgiani has focused his career primarily on the special problems of coccidioidomycosis (Valley Fever) and its impact on the general population and special groups such as organ transplant recipients and patients with AIDS. For 19 years, he was project director of an NIH-sponsored coccidioidomycosis clinical trials group. Dr. Galgiani's laboratory has collaborated in efforts to develop vaccines to prevent Valley Fever. For the past 5 years, Dr. Galgiani has led a development program for nikkomycin Z, a possible cure for Valley Fever, now in clinical trials. In 1996, Dr. Galgiani founded the Valley Fever Center for Excellence to disseminate information about Valley Fever, help patients with the severest complications of this disease, and encourage research into the biology and diseases of its etiologic agent.

Julie Harris, Ph.D., M.P.H., received her bachelors' degree from Rensselaer Polytechnic Institute, her Ph.D. in microbiology from Columbia University and her M.P.H. from Johns Hopkins in epidemiology before joining the CDC's Epidemic Intelligence Service in 2007, where she worked on the prevention and control of enteric infections in the United States and in several countries in Africa.

She joined the Mycotic Diseases Branch at the CDC in 2009, where her first task was to create a surveillance system for *Cryptococcus gattii*, an emerging fungal infection in the Pacific Northwest. She also works on mycotoxins in Bangladesh and Guatemala, cryptococcal infections in Thailand, and coccidioidomycosis in the United States.

Joseph Heitman, M.D., Ph.D., is chair and James B. Duke Professor, Department of Molecular Genetics and Microbiology, Duke University. He received his B.S. and M.S. in chemistry and biochemistry at the University of Chicago, and M.D. and Ph.D. from Cornell and Rockefeller Universities. He was an EMBO fellow at the Biocenter in Switzerland where he pioneered yeast as a model to study immunosuppressive drugs. He elucidated the role of FKBP12 in forming complexes with FK506 and rapamycin that inhibit cell signaling and growth and discovered the targets of rapamycin TOR1/TOR2, pathways conserved from yeasts to humans. Dr. Heitman has been at Duke since 1992, and focuses on model and pathogenic fungi, studying the evolution of sex and roles of sexual reproduction in microbial pathogens; how cells sense and respond to nutrients and the environment; the targets and mechanisms of action of immunosuppressive and antimicrobial drugs; and the genetic and molecular basis of microbial pathogenesis and development. Their discovery of fungal unisexual mating has implications for how sex might create diversity de novo with implications for pathogen evolution and emergence.

Dr. Heitman received the Burroughs Wellcome Scholar Award in Molecular Pathogenic Mycology, the ASBMB AMGEN award, and the Infectious Diseases Society of America (IDSA) Squibb Award. He has taught the Molecular Mycology Course at the MBL since 1998. He is an editor for *Eukaryotic Cell*, *Fungal Genetics and Biology*, and *PLoS Pathogens*; a board member for *PLoS Biology*, *Current Biology*, and *Cell Host & Microbe*; an advisory board member for the Broad Institute Fungal Genome Initiative and the Department of Energy/JGI Fungal Kingdom project; and cochair/chair for the FASEB Microbial Pathogenesis conference (2011, 2013). He is a Fellow of the American Society for Clinical Investigation, the IDSA, the American Academy of Microbiology, AAAS, and the Association of American Physicians.

Steven Holland, M.D., received his M.D. and Medicine and Infectious Disease training from Johns Hopkins. He came to NIH in 1989 as a National Research Council fellow in the Laboratory of Molecular Microbiology, working on transcriptional regulation of HIV. In 1991, Dr. Holland joined the Laboratory of Host Defenses, shifting his research to the host side, with a focus on phagocyte defects and their associated infections. His work centered on the pathogenesis and management of chronic granulomatous disease, as well as other congenital immune defects affecting phagocytes, including those predisposing to mycobacterial and fungal diseases. In 2004, he became chief of the Laboratory of Clinical

Infectious Diseases. The laboratory takes a fully integrated approach to infectious disease, incorporating the molecular genetics of the host and the pathogen as well as mechanisms of pathogenesis that allow the development and study of novel therapeutics. The integrated bench-to-bedside model adds clinical insight into mechanisms of action and therapy. The laboratory has been engaged in the human genetics of fungal susceptibility for several years, identifying specific Mendelian associations with genes in the NADPH, STAT3, DOCK8, and interferon gamma/IL-12 pathways.

Mogens Støvring Hovmøller, Ph.D., is senior plant pathologist at Aarhus University, Faculty of Agricultural Sciences, Department of Integrated Pest Management in Denmark. He completed his Ph.D. in 1991 at the Royal and Veterinary and Agricultural University (now Copenhagen University) in population genetics of fungal crop pathogens. His research interests expand from host–pathogen and pathogen–environment interactions to population genetics, evolutionary biology, and epidemiology of crop pathogens. He has been involved in multiple international research projects focusing on dispersal and evolution of *Puccinia striiformis*, a basidiomycete fungus causing yellow rust on wheat. He is a board member of the European and Mediterranean Cereal Rust Foundation and member of the Technical Advisory Committee of the Borlaug Global Rust Initiative. He is leading a Global Rust Reference Center (GRRC) located in Denmark, which was launched in 2009 by Aarhus University, the International Center for Agricultural Research in the Dry Areas (ICARDA), and the International Maize and Wheat Improvement Center (CIMMYT). GRRC is complementing existing wheat rust surveillance efforts by the Food and Agricultural Organisation of the United Nations, CIMMYT, ICARDA, and national rust diagnostic laboratories in Europe, Australia, North America, and elsewhere, and extends and maintain a wheat rust gene bank to support international resistance breeding and research.

Barbara Howlett, Ph.D., has a B.Sc. (Honors) in biochemistry and a Ph.D. in botany from University of Melbourne, Australia. She has also spent time at the Australian National University, Canberra, University of California, Berkeley, and Stanford University. She has worked in a diverse range of research areas, including immunology, memory in bacteria chemotaxis, nitrogen fixation, and plant disease. Her current research is on blackleg, the major disease of the oilseed crop, canola. Her approach to this topic is multidisciplinary and holistic, ranging from developing disease management strategies for farmers, to coleading an initiative to sequence and annotate the genome of the blackleg fungus. She is also interested in parallels and differences between fungal pathogenesis of plants and animals. These experiences and her membership on a panel of the Australian Grains Research and Development Corporation, which invests \$AUD120 million pa into grains research, have familiarized her with disease threats to agriculture and food production. Dr. Howlett has published more than 110 refereed scientific articles.

She is an associate editor of *PLoS Pathogens*, a senior editor of *Molecular Plant Pathology*, and on the editorial board of *Eukaryotic Cell*.

Mike Jeger, Ph.D., is a professor in the Division of Biology, Imperial College London, based at the Silwood Park campus, near Ascot, U.K. His research interests are in quantitative plant disease epidemiology, mathematical modeling, and disease management. He has worked on a wide range of plant–pathogen systems, including fungal (and other) pathogens of agricultural and horticultural crops, forest trees, and plants in natural grassland communities and has published extensively in the related scientific literature. He currently works on theoretical models concerning the spread of exotic plant pathogens in networks, such as in the horticultural nursery trade, where invasion criteria and the potential size of disease outbreaks depend critically on network structure. He is involved professionally and internationally in issues relating to plant health and is currently chair of the Plant Health Panel of the European Food Standards Agency, which provides independent advice to the European Commission. He will soon take up an emeritus professorship and become a senior research investigator in the Centre for Environmental Policy at Imperial College, where he will work on policy and technical issues relating to biosecurity.

Lawrence C. Madoff, M.D., serves as director of epidemiology and immunization for the Massachusetts Department of Public Health, where he oversees programs related to infectious disease threats in the commonwealth. He is an academic infectious disease physician specializing in the epidemiology of emerging pathogens, bacterial vaccine development, and international health. He is a professor of medicine at the University of Massachusetts Medical School and is on the attending staff at University of Massachusetts Memorial Medical Center. In addition, Dr. Madoff has been the editor of ProMED-mail, the Program for Monitoring Emerging Diseases, since 2002. In this capacity, he has expanded the program to more than 50,000 participants and extended its reach through the development of regional projects in the Mekong Basin, Africa, and the newly independent states of the former Soviet Union. Dr. Madoff chaired the organizing committees for the International Meetings on Emerging Diseases and Surveillance in 2007, 2009, and 2011. He is a member of the American Society for Microbiology, the International Society for Infectious Diseases, and the Council of State and Territorial Epidemiologists; past president of the U.S. Lancefield Streptococcal Research Society; and a Fellow of the IDSA and the American College of Physicians.

Luis Padilla, D.V.M., has served as the staff clinical veterinarian for the Smithsonian Conservation Biology Institute of the National Zoological Park in Front Royal, Virginia, since 2007. He received his Doctorate of Veterinary Medicine from Cornell University, where he also obtained a B.S. degree in biology. Dr.

Padilla completed postdoctoral clinical training at the Oradell Animal Hospital in New Jersey and at the Saint Louis Zoo, in Missouri. After serving as associate veterinarian at the Oklahoma City Zoo and as adjunct professor of zoological medicine at Oklahoma State University, Dr. Padilla joined the Smithsonian's National Zoo as a supervisory veterinarian in 2006. His academic interests are in the anesthesia of wildlife and non-domestic species, ungulate medicine and advanced disease diagnostics, and the use of captive animals as models to understand disease dynamics in free-ranging populations. He serves as the veterinary advisor for the clouded leopard species survival plan and is a Diplomate of the American College of Zoological Medicine.

David Rizzo, Ph.D., received his Ph.D. in plant pathology from the University of Minnesota and joined the faculty of the University of California-Davis, Department of Plant Pathology and the Graduate Group in Ecology in 1995. Research in his lab focuses on the ecology and management of forest tree diseases, including diseases caused by both native and introduced pathogens. Research in the lab takes a multiscale approach ranging from experimental studies on the basic biology of organisms to field studies across forest landscapes. Active collaborations include projects with landscape ecologists, epidemiologists, molecular biologists, entomologists, and forest managers. The primary research effort in the lab is currently *Phytophthora* species in California coastal forests, with an emphasis on sudden oak death. In conifer forests of the Sierra Nevada Mountains, the lab studies a variety of diseases and their relationship to past and present forest management and conservation issues. In addition to research, Dr. Rizzo teaches undergraduate and graduate courses in mycology. Since 2004, he has been director of the Science and Society program in the College of Agricultural and Environmental Sciences. Dr. Rizzo also serves as the scientific advisor for the California Oak Mortality Task Force.

Erica Bree Rosenblum, Ph.D., is an evolutionary biologist focused on understanding the molecular mechanisms of evolution. She is currently an assistant professor in the Department of Biological Sciences at the University of Idaho. Dr. Rosenblum's research emphasizes understanding the processes that generate and impact biological diversity (i.e., speciation and extinction). She uses genetic and genomics tools in the lab, but also works with endangered species in their natural habitats. Much of her current work focuses on understanding mechanisms of host-pathogen interaction between frogs and the chytrid fungus responsible for amphibian declines. She collaborates with an international, multidisciplinary working group to understand the catastrophic impacts of this emerging fungal pathogen. In addition to her research, Dr. Rosenblum contributes to a variety of educational initiatives, and her work has been featured in a number of forums, including the *New York Times*, the Discovery Channel, *Science*, *Ranger Rick*, and *Natural History Magazine*. Dr. Rosenblum received her B.A. from the Ecology

and Evolutionary Biology Department at Brown University and completed her Ph.D. in the Integrative Biology Department at the University of California–Berkeley. She conducted her postdoctoral research as an NSF Bioinformatics Fellow in the Department of Genome Sciences at the Lawrence Berkeley National Laboratory. Her work is funded by the NSF and NIH.

Jim Stack, Ph.D., is director of the Great Plains Diagnostic Network (GPDN) and a professor of plant pathology at Kansas State University. As director of GPDN, Dr. Stack coordinates a nine-state project for the rapid detection and accurate diagnosis of high-consequence pathogens and pests. He is the Principal Investigator of a plant biosecurity project at the National Agriculture Biosecurity Center and has collaborated on several international projects regarding plant biosecurity. Prior to joining Kansas State, Dr. Stack was on the faculty at the University of Nebraska and at Texas A&M University. He formerly worked for *EcoScience Corporation* as the director of applied research, leading the discovery, development, and commercialization of microbe-based products to protect fruit from storage decay pathogens. His research interests include pathogen detection and diagnostics, pathogen ecology, and epidemiology.

Compton Tucker, Ph.D., is a senior earth scientist in the Biospheric and Hydrospheric Sciences Laboratory at the National Aeronautics and Space Administration's (NASA's) Goddard Space Flight Center in Maryland. A native of Carlsbad, New Mexico, he holds a B.S. degree in biology and M.S. and Ph.D. degrees in forestry, all from Colorado State University. Upon completing his B.S. in biology, he worked at Colorado National Bank in Denver and the First National Bank of Albuquerque, New Mexico. Realizing that banking was not his calling, he entered graduate school at Colorado State University and was associated with the Natural Resource Ecology Laboratory for his graduate work. After completing his Ph.D. degree in 1975, he was a National Academy of Sciences Postdoctoral Fellow at the Goddard Space Flight Center before joining NASA as a physical scientist. He is the author of approximately 165 journal articles on the use of remote sensing to study vegetation that have been cited more than 14,000 times. In collaboration with coworkers, he is presently studying tropical deforestation and fragmentation, global variations in photosynthetic capacity, climatically coupled diseases, tropical glacier variation from Bolivia to Mexico, and climate using satellite and ground data. He was on NASA detail to the Climate Change Science Program from 2006 to 2009. He is the recipient of NASA's Exceptional Scientific Achievement Medal, the Henry Shaw Medal from the Missouri Botanical Garden, National Air and Space Museum Trophy, the William Nordberg Memorial Award for Earth Sciences, the Mongolian Friendship Medal, the William T. Pecora Award from the U.S. Geological Survey, and the Galathea Medal from the Royal Danish Geographical Society. He is a fellow of the American Geophysical Society.

Vance Vredenburg, Ph.D., is an assistant professor of biology at San Francisco State University. His research includes studies on the ecology, evolution, and conservation of amphibians. His current research investigates the impacts of emerging infectious disease on amphibian hosts. With a collaborative team, he studies chytridiomycosis, the lethal amphibian disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), which is implicated in mass die-offs of amphibians globally. Dr. Vredenburg and colleagues recently documented the spread of *Bd* through susceptible frog populations in the protected parks of the Sierra Nevada, California. While most populations are driven completely to extinction after pathogen arrival, a few populations survive. His most recent work investigates the role that symbiotic skin microbes (e.g., *Janthinobacterium lividum*, a bacterium) may play in frog host immunity.

Dr. Vredenburg received his Ph.D. from the University of California, Berkeley, in 2002. He is a cofounder of AmphibiaWeb (www.AmphibiaWeb.org), an online conservation resource for the world's amphibians that receives an average of more than 20,000 successful searches per day from students, research biologists, and conservationists worldwide. Dr. Vredenburg is a research associate at the California Academy of Sciences and the Museum of Vertebrate Zoology. His research is funded by the NSF.

Ché Weldon, Ph.D., M.Sc., holds research interests that have always been centered on amphibians, which started in 1997 with host–parasite interactions (B.Sc. Honors project) and was followed by an in-depth assessment of the sustainable global use of African clawed frogs (M.Sc. project, 1998–1999). The following year, as a field biologist with the Southern African Frog Atlas and Red Data Project, Dr. Weldon developed a more focused interest in amphibian conservation. It was during this time that the amphibian chytrid fungus was first detected and a global surge started to investigate its role in amphibian declines. For his studies on amphibian chytrid in South Africa, Dr. Weldon received the W.O. Neitz medal for best Ph.D. thesis in parasitology. With then-supervisor L. H. du Preez, Dr. Weldon established the African Amphibian Conservation Research Group. Dr. Weldon specialized in amphibian diseases in Africa as a Postdoctoral Fellowship at North-West University, and later became a zoology lecturer at the university where he has continued his research on the amphibian chytrid and amphibian conservation. From the 12 peer-reviewed articles that Dr. Weldon has authored in the past 6 years, 6 have been cited a total of 130 times.

Gudrun Wibbelt, D.V.M., M.R.C.V.S., graduated from University of Veterinary Medicine Hannover, Germany, followed by a residency for veterinary pathology at University of Liverpool, United Kingdom. Since 2005 she is a certified veterinary pathologist with a special focus on wildlife and zoo animal diseases. She heads the wildlife pathology and electron microscopy unit of the Leibniz Institute for Zoo and Wildlife Research, Berlin, which has a unique standing in

wildlife and nature conservation research in Germany/Europe. For more than 5 years, one of her main research interests has been the diseases of European bats, with special emphasis on the correlation of histopathology and bacteriology/virology, an aspect largely neglected by investigations on bats and emerging diseases. With the emergence of white-nose syndrome of bats in the United States, she leads collaborative European research efforts on fungal infections in native bats from Europe.

